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Klatzmann et al.

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(54) **PROTOZOAN VARIANT-SPECIFIC SURFACE PROTEINS (VSP) AS CARRIERS FOR ORAL DRUG DELIVERY**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(22) Filed: **Mar. 15, 2013**

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C12Q 1/00	(2006.01)
A61K 38/28	(2006.01)
C07K 14/62	(2006.01)
A61K 38/00	(2006.01)
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A61P 5/06	(2006.01)
A61P 3/10	(2006.01)
A61P 7/12	(2006.01)
A61K 45/00	(2006.01)
A61K 47/00	(2006.01)
A61K 39/00	(2006.01)
A61K 47/42	(2006.01)
C07K 14/44	(2006.01)
A61K 47/48	(2006.01)
A61K 39/39	(2006.01)
A61Q 19/00	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 47/42*** (2013.01); ***A61K 38/27*** (2013.01); ***A61K 38/28*** (2013.01); ***A61K 47/48246*** (2013.01); ***C07K 14/44*** (2013.01); *A61K 38/00* (2013.01); *A61K 38/26* (2013.01); *A61K 39/39* (2013.01); *A61Q 19/00* (2013.01); *C07K 2319/02* (2013.01); *C07K 2319/21* (2013.01)

(58) **Field of Classification Search**

CPC A61K 38/28; A61K 38/00; A61K 38/27; A61K 38/26; A61K 39/39; A61Q 19/00; C07K 14/44

See application file for complete search history.

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Primary Examiner — Gary Nickol
Assistant Examiner — Lakia Tongue
(74) *Attorney, Agent, or Firm* — Sterne, Kessler, Goldstein & Fox P.L.L.C.

(57) **ABSTRACT**

The invention provides compositions for oral delivery and methods of treatment using VSP carriers, such as *Giardia* sp. variable surface proteins (VSP), to deliver therapeutic agents. VSP drug carriers can be combined with bioactive peptides, e.g., insulin, glucagon, or hGH, and be administered orally or mucosally. VSP carriers are resistant to acidic pHs and to proteolytic degradation and protect therapeutic agents from degradation in the gastrointestinal tract.

36 Claims, 12 Drawing Sheets

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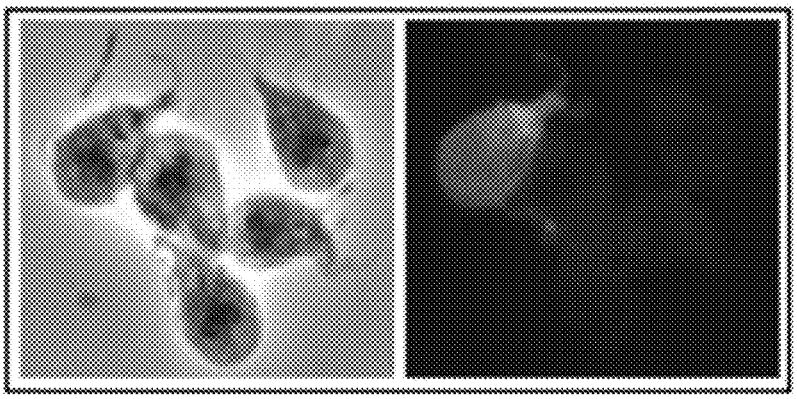
A



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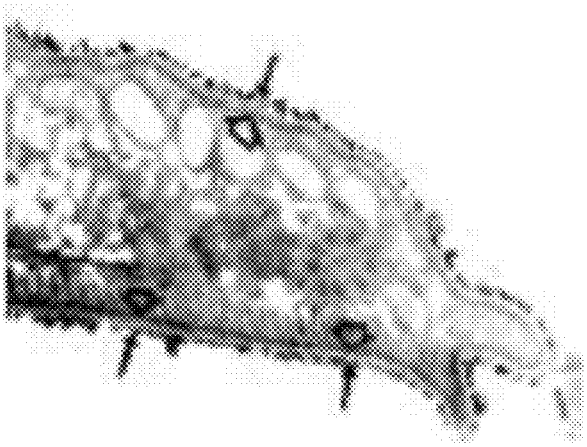


C

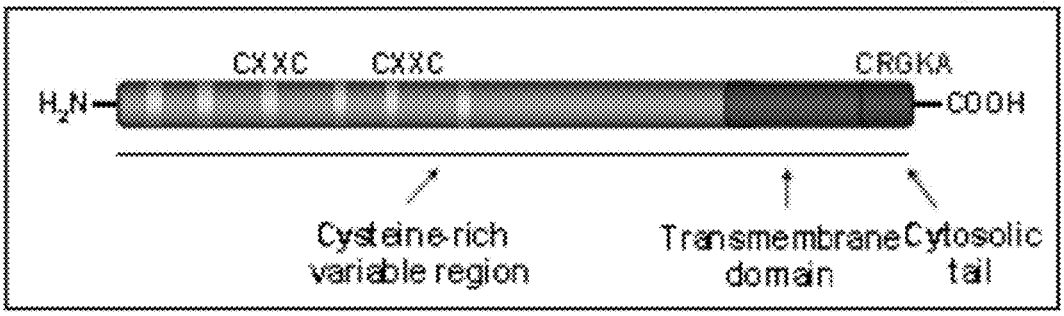

CXXC
CXXC
CRGKA
H2N
COOH
Cysteine-rich variable region
Transmembrane domain
Cytosolic tail

FIG. 1

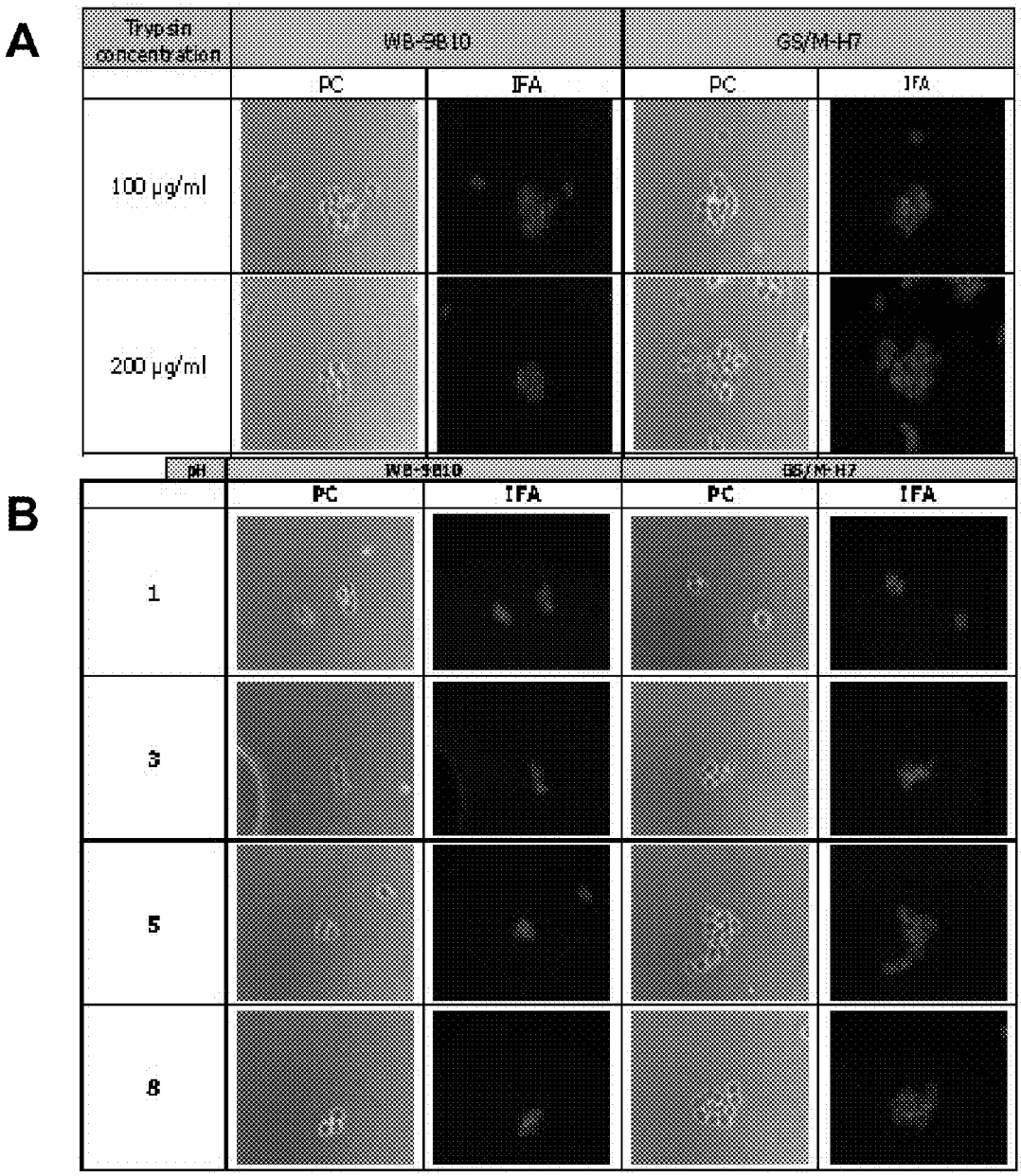

A
Trypsin concentration
WB-9B10
GS/M-H7
PC
IFA
PC
IFA
100 μg/ml
200 μg/ml
B
pH
WB-9B10
GS/M-H7
PC
IFA
PC
IFA
1
3
5
8

FIG. 2

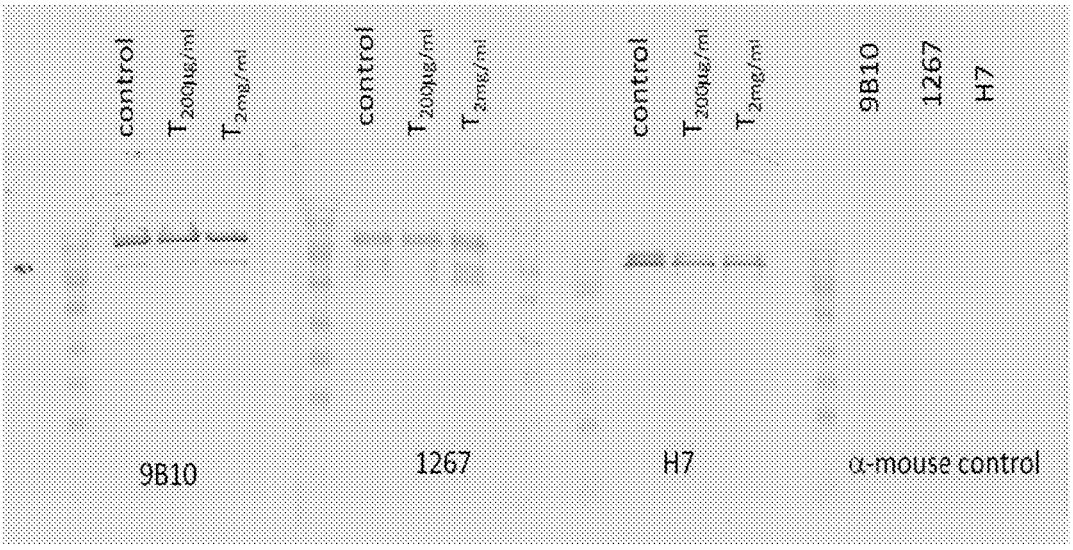

control
T 200μg/ml
T 2mg/ml
control
T 200μg/ml
T 2mg/ml
control
T 200μg/ml
T 2mg/ml
9B10
1267
H7
9B10
1267
H7
α-mouse control

FIG. 3

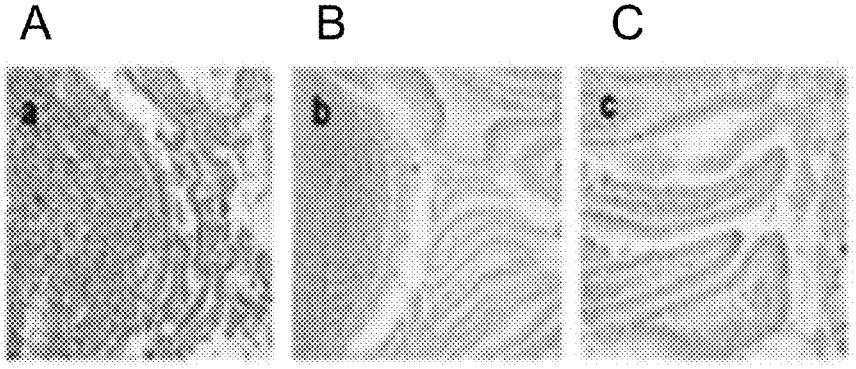

A
B
C
a
b
c

FIG. 4

A

MLLIAFYLILSTFAVDCKNSGNSCEAGQCDTIGDTEICMQCNQGKVPINGICTAHSEEAVTNAGCKKNGGTNIEESDK
VCGQCGNGYFLHKGGCYKIGEAPGNLICADEASNPGARTAGVCGACKDGYYKNSDAVATADSCIACEDANCATCGGAG
ENKCTKCIDGYFVGATGNEGGCIKCDATTGPNSYKGVAGCARCEKPKNAGPAKCIECAADYLKTEADEQTSCVSEAVC
REGKTHFPTTDSAGGNKKVCVSCGTTNNGGIENCGECTSKESAARAGTEITCTKCSSNNLSPLGDACLTDCPAGTYAV
SGDSGSVCKPCHNTCAGCQTDDRETSCTACSPGYSLLYESNGATGRCVKECTGAFITNCADGQCTANVGGAKYCTQCK
DGYAPIDGICTAVAAAGRDVSVCTATGGKCTACTGNYALLSGGCYNTQTLPGKSVCKAVANSNDGKCKTCANGQAPDP
ATNFCPLCDSTCAECSTKNDADACTKCFPGYYKTGNKCIKCTESSNNGKKIDGIPDCLSCEAPINTGPAICYVKTDGT
SDDNSGNGGDSTNKSGLSTGHHHHHH

(Recombinant VSP1267, SEQ ID NO: 1)

B

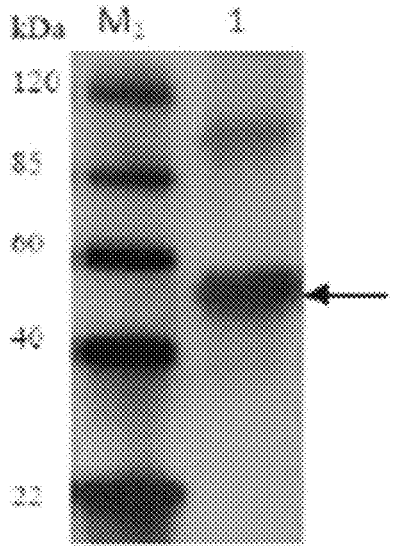


FIG. 5

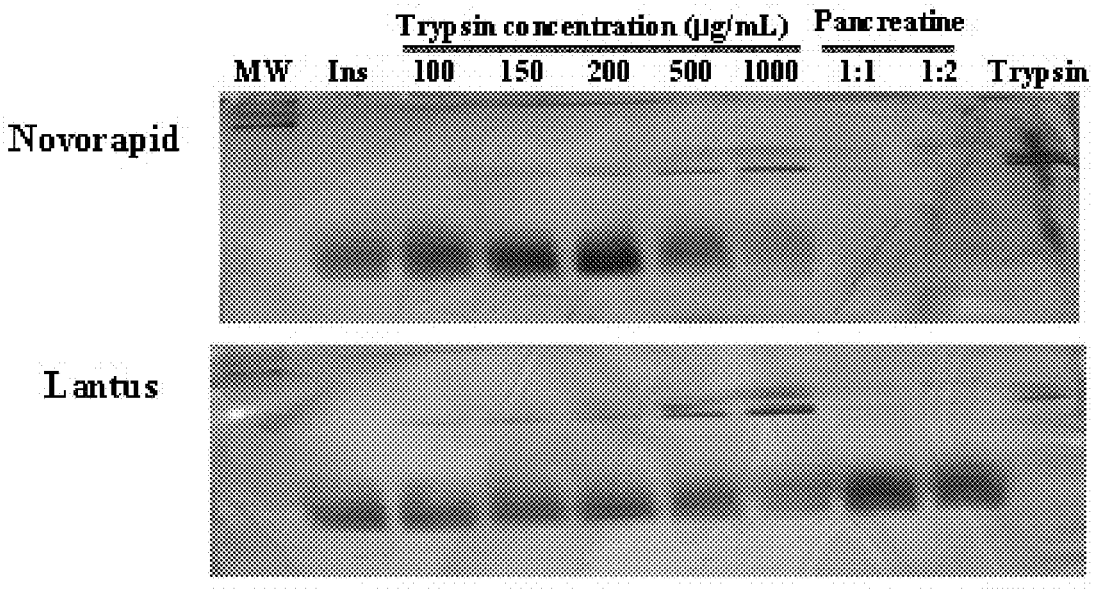
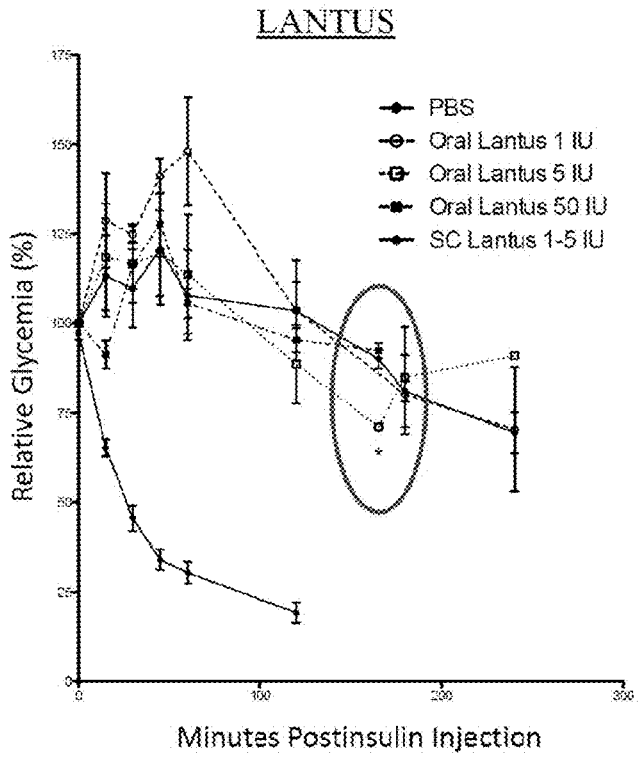

Trypsin concentration (μg/mL)
Pancreatine
MW
Ins
100
150
200
500
1000
1:1
1:2
Trypsin
Novorapid
Lantus

FIG. 6

A


LANTUS
PBS
Oral Lantus 1 IU
Oral Lantus 5 IU
Oral Lantus 50 IU
SC Lantus 1-5 IU
Relative Glycemia (%)
Minutes Postinsulin Injection

B

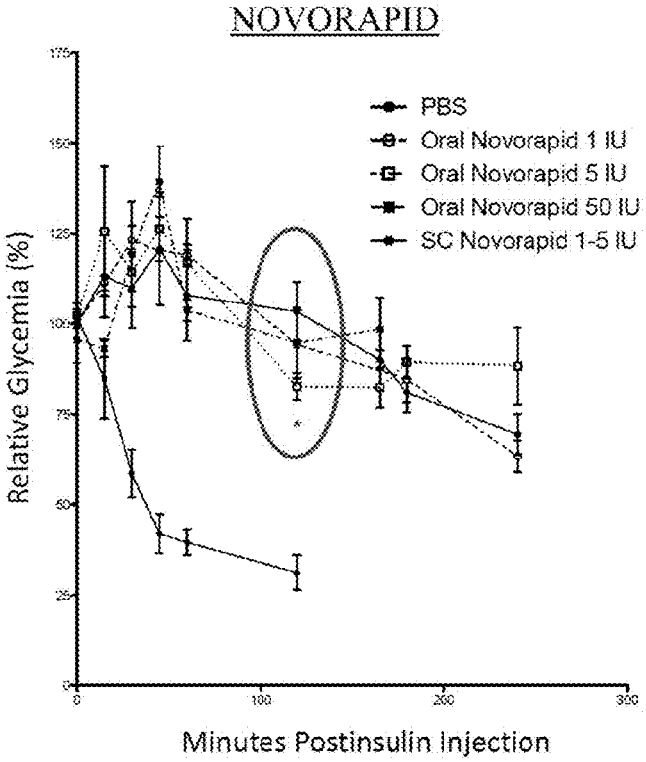
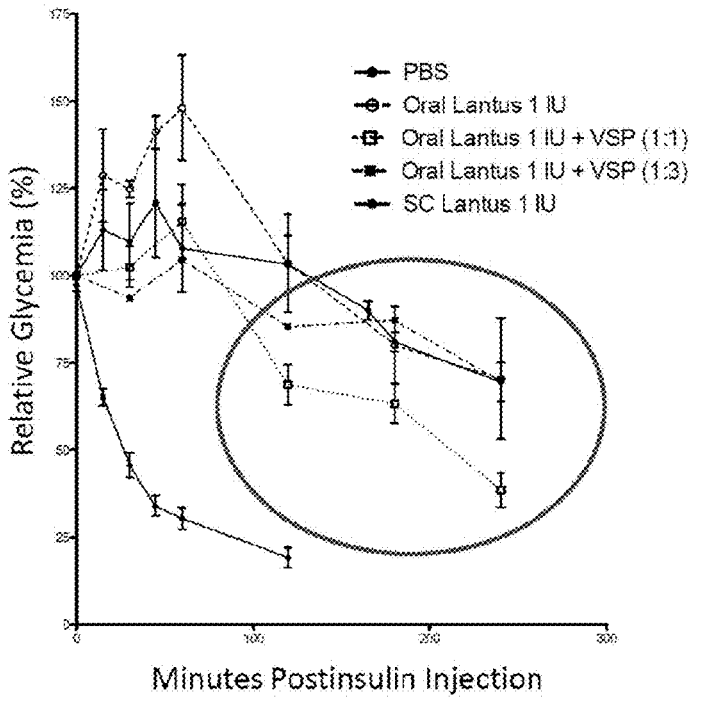

NOVORAPID
PBS
Oral Novorapid 1 IU
Oral Novorapid 5 IU
Oral Novorapid 50 IU
SC Novorapid 1-5 IU
Relative Glycemia (%)
Minutes Postinsulin Injection

FIG. 7

A


PBS
Oral Lantus 1 IU
Oral Lantus 1 IU + VSP (1:1)
Oral Lantus 1 IU + VSP (1:3)
SC Lantus 1 IU
Relative Glycemia (%)
Minutes Postinsulin Injection

B

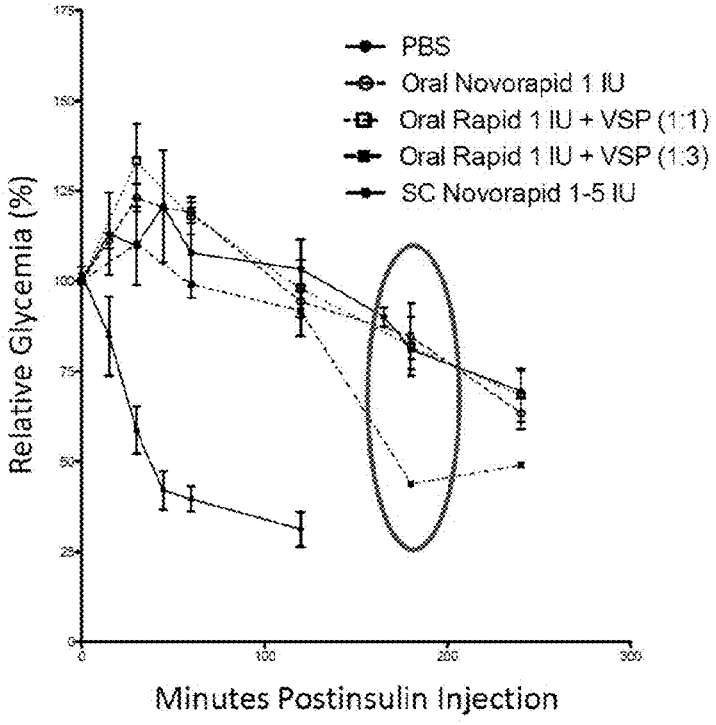

PBS
Oral Novorapid 1 IU
Oral Rapid 1 IU + VSP (1:1)
Oral Rapid 1 IU + VSP (1:3)
SC Novorapid 1-5 IU
Relative Glycemia (%)
Minutes Postinsulin Injection

FIG. 8

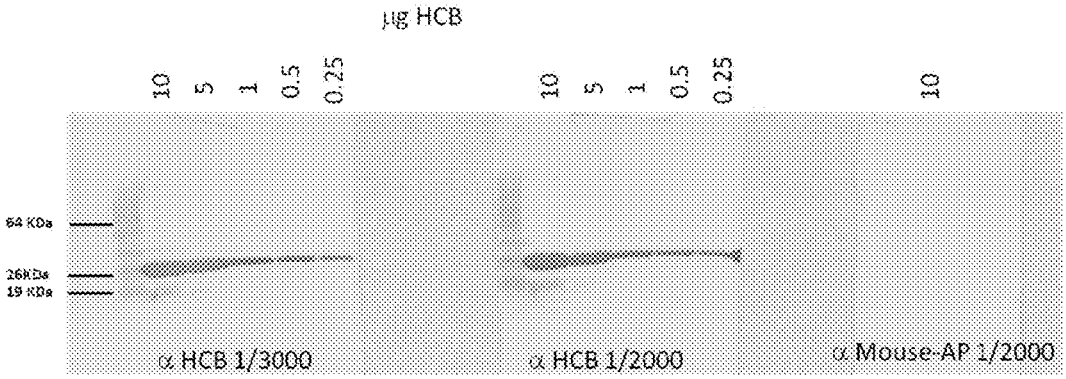

µg HCB
10
5
1
0.5
0.25
10
5
1
0.5
0.25
10
64 KDa
26KDa
19 KDa
α HCB 1/3000
α HCB 1/2000
α Mouse-AP 1/2000

FIG. 9

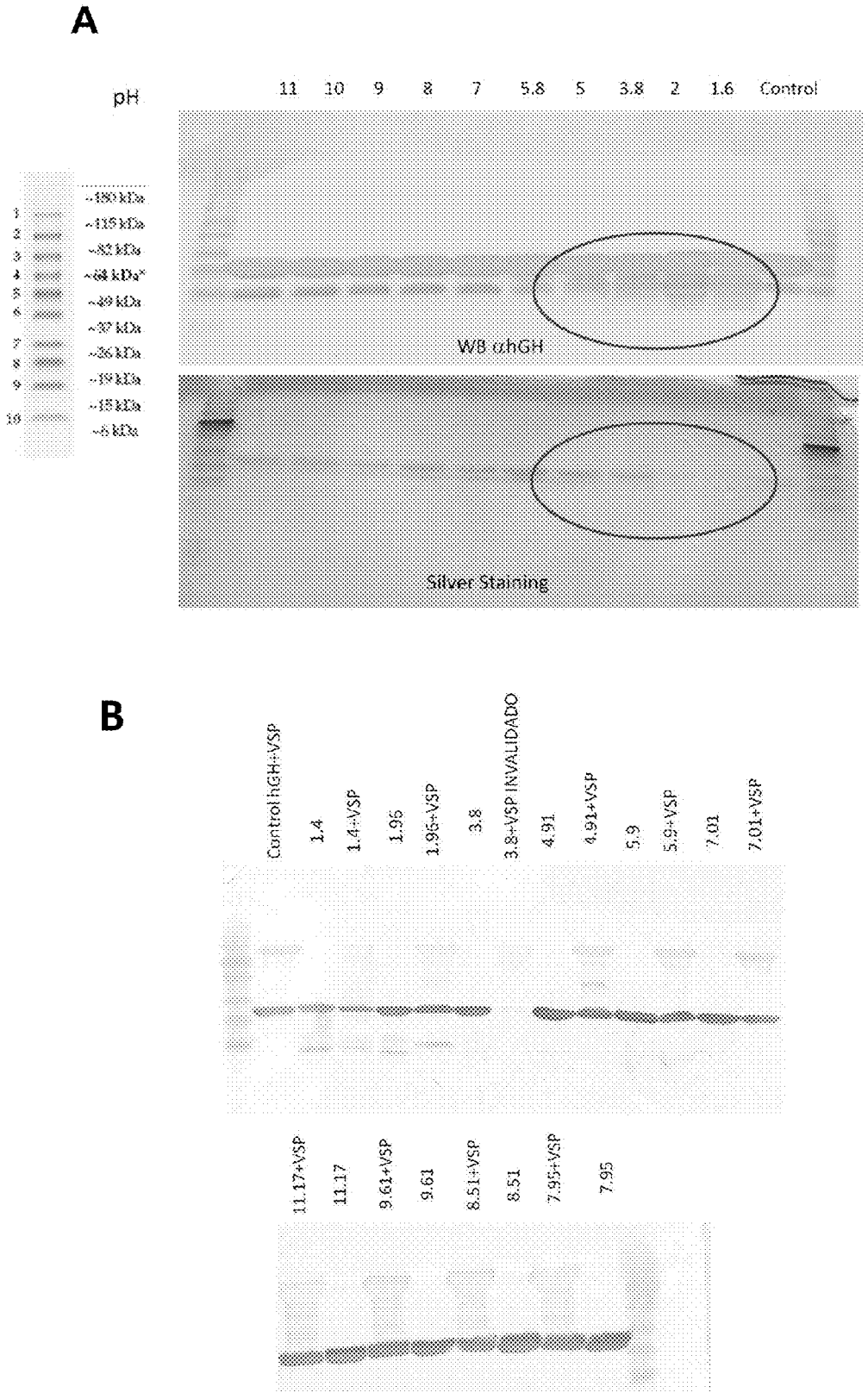

A
pH
11 10 9 8 7 5.8 5 3.8 2 1.6 Control
~180 kDa
~116 kDa
~82 kDa
~64 kDa*
~49 kDa
~37 kDa
~26 kDa
~19 kDa
~15 kDa
~6 kDa
WB αhGH
Silver Staining
B
Control hGH+VSP
1.4
1.4+VSP
1.95
1.95+VSP
3.8
3.8+VSP INVALIDADO
4.91
4.91+VSP
5.9
5.9+VSP
7.01
7.01+VSP
11.17+VSP
11.17
9.61+VSP
9.61
8.51+VSP
8.51
7.95+VSP
7.95

FIG. 10

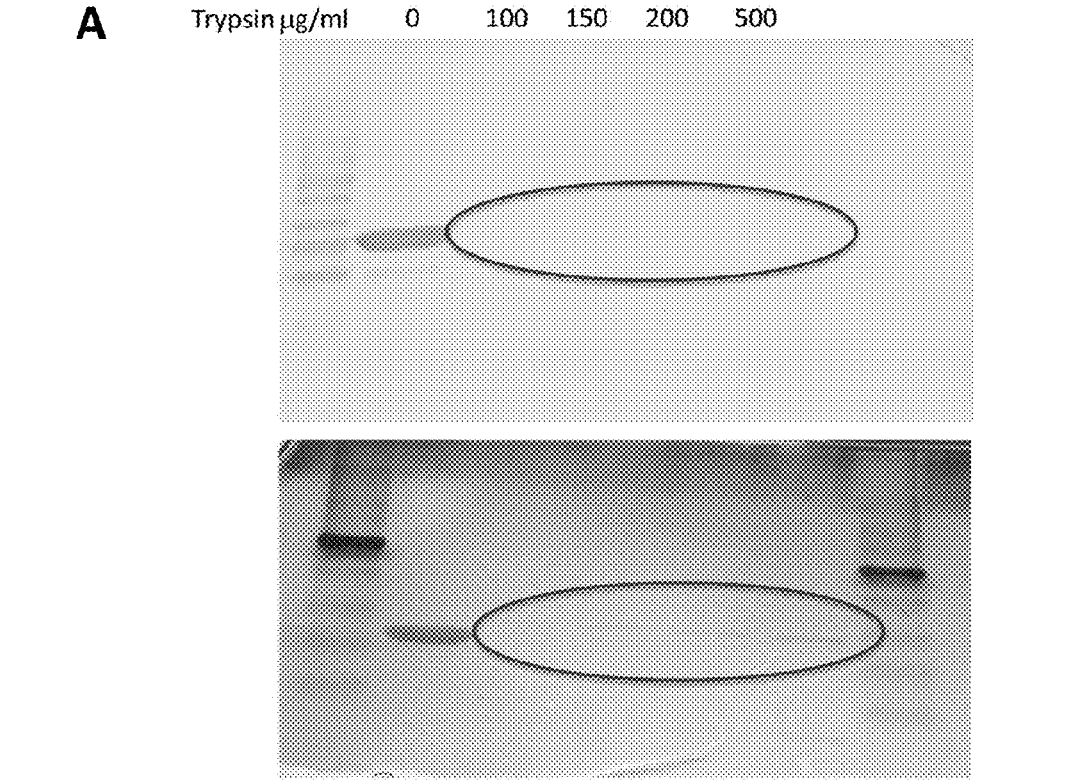
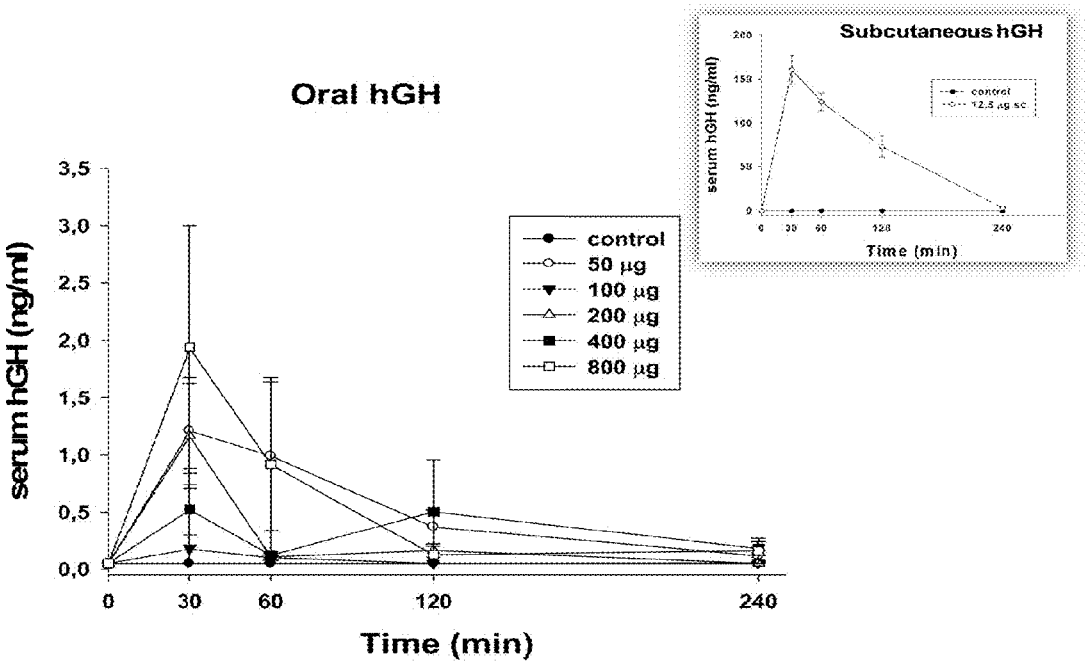

A
Trypsin μg/ml 0 100 150 200 500
B
Trypsin
500 μg/ml + VSP
500 μg/ml
100 μg/ml + VSP
100 μg/ml
Control hGH+VSP
300 μg/ml + VSP
300 μg/ml
150 μg/ml + VSP
150 μg/ml
100 μg/ml + VSP
100 μg/ml
50 μg/ml + VSP
50 μg/ml
Control hGH+VSP

FIG. 11

A


Oral hGH
serum hGH (ng/ml)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
30
60
120
240
Time (min)
control
50 µg
100 µg
200 µg
400 µg
800 µg
Subcutaneous hGH
serum hGH (ng/ml)
Time (min)

B

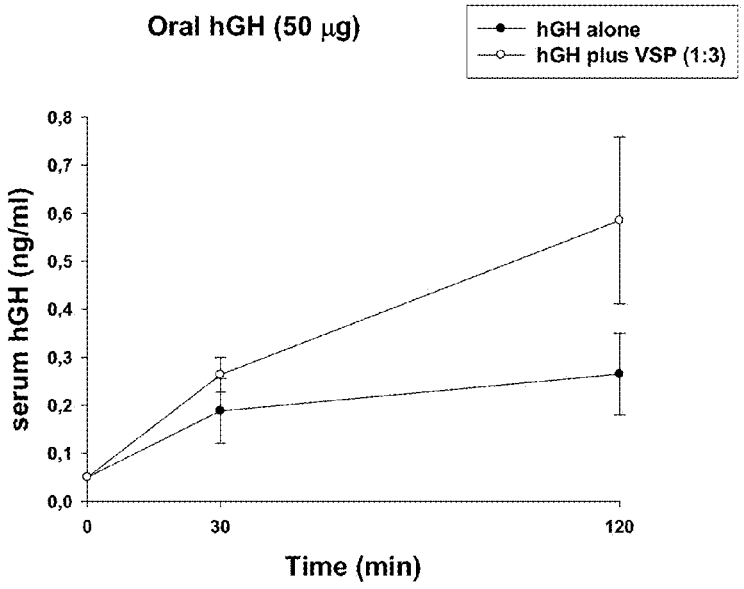

Oral hGH (50 µg)
hGH alone
hGH plus VSP (1:3)
serum hGH (ng/ml)
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
0
30
120
Time (min)

FIG. 12

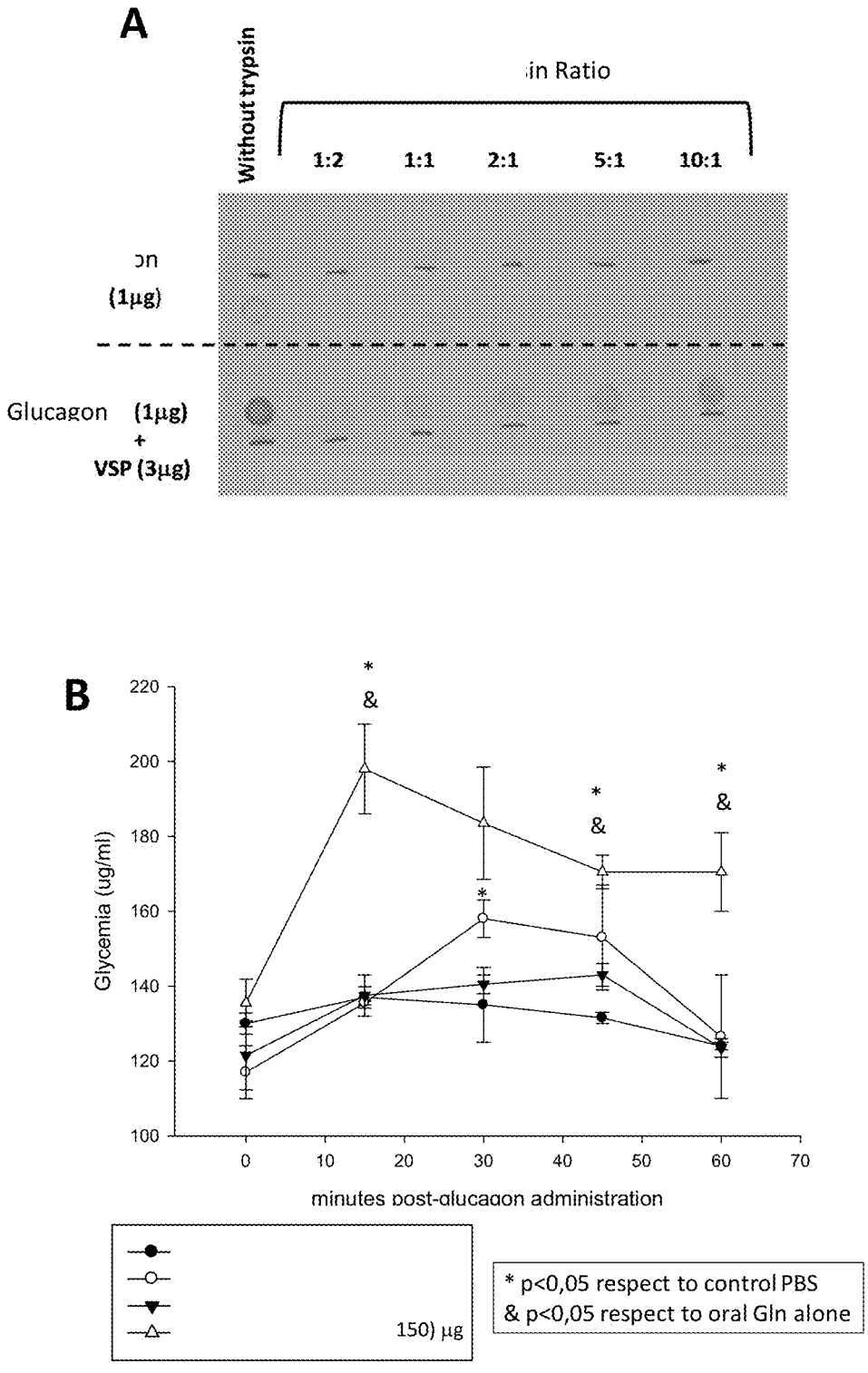

A
Without trypsin
in Ratio
1:2
1:1
2:1
5:1
10:1
on
(1µg)
Glucagon (1µg)
+
VSP (3µg)
B
Glycemia (ug/ml)
220
200
180
160
140
120
100
0
10
20
30
40
50
60
70
minutes post-glucagon administration
150) µg
* p<0,05 respect to control PBS
& p<0,05 respect to oral Gln alone

FIG. 13

PROTOZOAN VARIANT-SPECIFIC SURFACE PROTEINS (VSP) AS CARRIERS FOR ORAL DRUG DELIVERY

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

The content of the electronically submitted sequence listing (Name: 3181_0010001_sequence_listing_ST25_ascii.txt; Size: 2,810,589 bytes, and Date of Creation: Oct. 1, 2014) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND

The present invention relates to compositions and methods to deliver therapeutic agents to a subject in need thereof using polypeptide carriers. More particularly, the present invention relates to polypeptides such as *Giardia* sp. variable surface proteins (VSPs) that act as carriers for the delivery of therapeutic agents such as bioactive peptides.

Oral delivery represents the ideal means of delivering prophylactic and therapeutic agents because of ease of administration, patient compliance, and cost. However, the oral route is also the most difficult because of the numerous barriers posed by the gastrointestinal tract (GIT). The main challenges are enzymatic degradation in the stomach and upper intestinal tract, and lack of sufficient permeability through the GIT. Low pH in the stomach can subject therapeutic agents to physical and chemical degradation. Physical degradation of peptides generally involves modification of the native structure of a protein to a higher-order structure which may be a result of adsorption, aggregation, unfolding, precipitation, and/or complete/partial degradation to its amino acidic components. Chemical degradation usually involves blond cleavage and leads to the formation of a new product.

Giardia is an intestinal pathogen which is capable of surviving the harsh environmental conditions in the stomach and the upper small intestine. Like many protozoan microorganisms, *Giardia* undergoes antigenic variation (see, e.g., Zambrano-Villa et al., Trends Parasitol. 18: 272-8 (2002)), a mechanism by which it continuously switches its major surface molecules allowing the parasite to evade the host's immune response and establish chronic and/or recurrent infections (see, e.g., Nash, Mol. Microbiol. 45:585-90 (2002)). These surface antigens belong to a family of Variant-specific Surface Proteins (VSPs), which are integral membrane proteins that cover the entire surface of trophozoites.

VSPs possess a cysteine-rich amino-terminal region, and a conserved carboxy-terminal domain including a transmembrane region and a short cytoplasmic tail (FIG. **1**C). There is a repertoire of about 200 VSP genes in the *Giardia*'s genome, but only one VSP is expressed on the surface of the parasite at any given time (see, e.g., Prucca et al., Nature 456(7223):750-4 (2008); Deitsch et al., Microbiol. Mol. Biol. Rev. 61:281-93 (1997)).

Since the extracellular portion of *Giardia* VSPs allows the parasite to survive within the hostile environment of the upper small intestine, VSPs covalently bound to antigens have been used to shuttle candidate antigens. It has been observed that when vaccines comprising VSPs covalently bound to *Giardia* antigens are administered orally, the vaccines fully protect animals from subsequent infections by the *Giardia* parasite, indicating that the antigens have survived the passage through the GIT (Rivero et al., Nat. Med. 16(5):551-7 (2010), see, e.g., PCT Pub. Nos. WP2010/064204 and WO2011/120994, which are herein incorporated by reference in their entireties).

Type 1 diabetes is usually diagnosed in children and young adults, and is responsible for a growing proportion of national health care expenditures. Type 1 diabetes is an autoimmune disease that results from a dysfunction of the immune system that attacks and destroys the β-cells of the pancreatic islets producing insulin.

Administration of exogenous insulin is the only medication that can be used to control the increases in blood sugar that occur with the disease. Type 2 diabetes, by contrast, is characterized by defects in both insulin secretion and insulin action, with insulin deficiency usually emerging later during the course of the disease. Insulin supplementation is often required to attain good glucose levels control in this disease (see, e.g., DeWitt & Hirsch, JAMA 289:2254-2264 (2003)). There are different types of subcutaneous insulin available (Summers et al., Clin. Ther. 26:1498-1505 (2004)). However, surveys indicate substantial resistance to insulin therapy on the part of patients with type 2 diabetes due to anticipated pain and inconvenience (Peyrot et al., Diabetes Care 28:2673-2679 (2005)). The youngest and oldest patients are least likely to accept injectable therapy and thus pose the greatest challenge for physicians who want to initiate insulin treatment (Freemantle et al., Diabetes Care 28:427-428 (2005)). Consequently, efforts to develop oral, nasal, and inhaled formulations of insulin have been driven by the preference of patients to avoid subcutaneous injections (Cefalu, Ann. Med. 33:579-586 (2001); Graham et al., N. Engl. J. Med. 356:497-502 (2007)). Thus, the option of delivering insulin by the oral route remains an attractive therapeutic strategy.

Glucagon, a peptide hormone secreted by the pancreas, raises blood glucose levels. Its effect is opposite that of insulin, which lowers blood glucose levels. Glucagon is indicated and used as a treatment for severe hypoglycemia. Because patients with type 1 diabetes may have less of an increase in blood glucose levels compared with a stable type 2 patient, supplementary carbohydrates should be given as soon as possible, especially to a pediatric patient. Glucagon is also indicated as a diagnostic aid in the radiologic examination of the stomach, duodenum, small bowel, and colon when diminished intestinal motility would be advantageous. Glucagon is as effective for this examination as are the anticholinergic drugs. However, the addition of the anticholinergic agent may result in increased side effects. As in the case of insulin, the development of forms of glucagon suitable for oral delivery is an attractive therapeutic strategy.

Growth hormone deficiency is a disorder that involves the pituitary gland, which produces growth hormone and other hormones. Human growth hormone (hGH) stimulates growth and cell reproduction in humans, also exerting its action on metabolism of lipids, proteins and carbohydrates. Recombinant hGH is commonly produced by bacterial fermentation (Zeisel et al., Horm. Res. 37(Suppl 2):5-13 (1992); Sonoda & Sigimura, Biosci. Biotechnol. Biochem. 72:2675-80 (2008)). When the pituitary gland does not produce enough growth hormone, growth will be slower than normal. Growth hormone is needed for normal growth in children. In adults, growth hormone is needed to maintain the proper amounts of body fat, muscle, and bone. hGH deficiency can occur at any age. Children and some adults with growth hormone deficiency will benefit from growth hormone therapy. To treat growth hormone deficiencies, hGH (human growth hormone) is generally prescribed. hGH

is an injectable drug which is injected underneath the fat of the patient's skin several times a week (Brearley et al., BMC Clin. Pharmacol. 7:10 (2007)). As in the case of insulin treatment, patient's resistance to the initiation injectable therapy and compliance pose challenges for physicians. Accordingly, the development of hGH forms suitable for oral delivery is an attractive therapeutic strategy.

BRIEF SUMMARY

The present disclosure provides compositions and methods comprising VSP-carriers for the delivery of therapeutic agents, e.g., bioactive peptides such as insulin, glucagon, or growth hormone, to a target location in a subject in need thereof, for example, via oral or mucosal administration.

More particularly, the disclosure provides the use of a VSP polypeptide, e.g., a *Giardia* parasite's variable surface protein (VSP) or a fragment thereof (e.g., the extracellular domain of a *Giardia* VSP or a CXXC (SEQ ID NO:589) motif-comprising fragment thereof) as a carrier to deliver a therapeutic agent via oral or mucosal administration. VSP carriers of the invention are not covalently bound to the therapeutic agents via peptidic bonds

Accordingly, the present disclosure provides a therapeutic composition comprising a VSP carrier and a therapeutic agent. In some embodiments, the composition is formulated for oral administration. In other embodiments, the composition is formulated for mucosal administration. In some embodiments, the VSP carrier is a VSP, a VSP-like protein, a VSP or VSP-like protein fragment, a VSP or VSP-like protein derivative, or a combination of two or more of said VSP carriers.

In some embodiments, the VSP carrier comprises a VSP from *Giardia* or a fragment thereof. In other embodiments, the VSP from *Giardia* or a fragment thereof comprises a VSP extracellular domain. In other embodiments, the VSP from *Giardia* is VSP1267. In some specific embodiments, the VSP carrier comprises the amino acid sequence of SEQ ID NO:2. In other embodiments, the VSP carrier further comprises a heterologous moiety. In some embodiments, the heterologous moiety is a protein purification tag sequence. In some embodiments, the protein purification tag sequence is a His6 tag. In some specific embodiments, the VSP carrier consists of the sequence of SEQ ID NO:1.

In some embodiments, the therapeutic agent is a biological agent. In some embodiments, the biological agent is a bioactive peptide. In some embodiments, the bioactive peptide is insulin, human growth hormone, glucagon, fragments, analogs, derivatives or variants thereof, or a combination of two or more of said bioactive peptides. In some embodiments, the bioactive peptide is a natural insulin. In other embodiments, the bioactive peptide is a recombinant insulin. In some embodiments, the bioactive peptide is an insulin analog. In other embodiments, the insulin analog is a fast-acting insulin. In other embodiments, the insulin analog is a long-acting insulin. In some embodiments, the fast-acting insulin is insulin aspart. In other embodiments, the long-acting insulin is insulin glargine.

In some embodiments, the molecule to molecule ratio of VSP carrier to the therapeutic agent ranges from about 10:1 to about 1:10. In other embodiments, the molecule to molecule ratio of VSP carrier to the therapeutic agent ranges from about 3:1 to about 1:3. In some embodiments, the molecule to molecule ratio of VSP carrier to the therapeutic agent is 3:1. In other embodiments, the molecule to molecule ratio of VSP carrier to the therapeutic agent is 1:1. In some embodiments, the composition further comprises a pharmaceutically acceptable excipient.

Also provided is a method of delivering a therapeutic agent to a target location in a subject comprising administering a therapeutic composition comprising a VSP carrier and a therapeutic agent to a subject in need thereof. The present disclosure also provides a method of treating a disease or condition in a subject comprising administering an effective amount of a therapeutic composition comprising a VSP carrier and a therapeutic agent to a subject in need thereof. In some embodiments, the disease or condition is a hormone deficiency. In some embodiments, the hormone deficiency is an insulin deficiency. In some embodiments, the insulin deficiency is type 1 diabetes.

Also provided is a method of treating a disease or condition in a subject comprising combining a VSP carrier and a therapeutic agent, where the VSP carrier binds to the therapeutic agent, and administering an effective amount of the combination of VSP carrier and therapeutic agent to the subject. The instant disclosure also provides a method of increasing the resistance of a therapeutic agent to enzymatic degradation comprising combining a VSP carrier and a therapeutic agent, where the VSP carrier can bind to the therapeutic agent, and wherein combining the VSP carrier and the therapeutic agent results in increased resistance of the therapeutic agent to enzymatic degradation.

The present disclosure also provides a method of increasing the resistance of a therapeutic agent to pH denaturation comprising combining a VSP carrier and a therapeutic agent, where the VSP carrier can bind to the therapeutic agent, and where combining the VSP carrier and the therapeutic agent results in increased resistance of the therapeutic agent to pH denaturation. Also provided is a method of increasing the attachability of a therapeutic agent to mucosal epithelial cells comprising combining a VSP carrier and a therapeutic agent, where the VSP carrier can bind to the therapeutic agent, and where combining the VSP carrier and the therapeutic agent results in increased attachability of the therapeutic to mucosal epithelial cells. In some embodiments, the mucosal epithelial cells are intestinal epithelial cells. In other embodiments, the mucosal epithelial cells are gastric epithelial cells. In some embodiments, the mucosal epithelial cells are oral epithelial cells.

Also provided is a method of making an orally deliverable composition, comprising combining a VSP carrier and a therapeutic agent, where the VSP carrier can bind to the therapeutic agent. The present disclosure also provides a method of making an injectable composition suitable for oral administration comprising combining a VSP carrier and a therapeutic agent, where the VSP carrier can bind to the therapeutic agent. For example, an injectable insulin composition can be reformulated or made suitable for oral administration by combining the injectable composition with a VSP carrier. In some embodiments, the VSP carrier is a VSP, a VSP-like protein, a VSP or VSP-like protein fragment, a VSP or VSP-like protein derivative, or a combination of two or more of said VSP carriers. In some embodiments, the VSP carrier comprises a VSP from *Giardia* or a fragment thereof. In other embodiments, the VSP from *Giardia* or a fragment thereof comprises a VSP extracellular domain. In some embodiments, the VSP from *Giardia* is VSP1267. In other embodiments, the VSP carrier comprises the amino acid sequence of SEQ ID NO:2. In some embodiments, the VSP carrier further comprises a heterologous moiety. In other embodiments, the heterologous moiety is a protein purification tag sequence. In some embodiments, the protein purification tag sequence is a His6

tag. In other embodiments, the VSP carrier consists of the sequence of SEQ ID NO:1. In some embodiments, the therapeutic agent is a biological agent. In other embodiments, the biological agent is a bioactive peptide. In some embodiments, the bioactive peptide is insulin, human growth hormone, glucagon, fragments, analogs, derivatives or variants thereof, or a combination of two or more of said bioactive peptides. In some embodiments, the bioactive peptide is a natural insulin. In other embodiments, the bioactive peptide is a recombinant insulin. In some embodiments, the bioactive peptide is an insulin analog. In other embodiments, the insulin analog is a fast-acting insulin. In some embodiments, the insulin analog is a long-acting insulin. In other embodiments, the fast-acting insulin is insulin aspart. In some other embodiments, the long-acting insulin is insulin glargine.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

FIG. **1**A shows phase contrast (left panel) and immunofluorescence (right panel) assays showing that a group of *Giardia* trophozoites. Each trophozoite expresses a single VSP on its surface, which is different for each trophozoite as demonstrated by surface labeling with an anti-VSP specific monoclonal antibody.

FIG. **1**B shows an anti-VSP specific immunogold labeling of the surface of a trophozoite. The entire surface of the parasite is labeled, including the ventral disk and the flagella, generating a thick surface coat.

FIG. **1**C is a diagram showing the structural characteristics of VSPs. The diagram shows that VSPs are integral membrane proteins with a variable extracellular region rich in CXXC (SEQ ID NO:589) motifs (where C indicates a cysteine and X can be any amino acid), a unique transmembrane hydrophobic regions and a short, 5 amino acids long cytoplasmic tail.

FIG. **2**A shows phase contrast (PC) images and immunofluorescent (IFA) images corresponding to two different *Giardia* isolates (WB and GS/M) treated with 100 μg/ml or 200 □g/ml trypsin. The monoclonal antibody G10/4 recognizes a conformational epitope in the VSPH7 VSP protein of the GS/M isolate. The monoclonal antibody 9B10 detects a non-conformational epitope in the VSP9B10 VSP protein of the WB isolate.

FIG. **2**B shows phase contrast (PC) images and immunofluorescent (IFA) images corresponding to two different *Giardia* isolates (WB and GS/M) incubated at different pHs (1, 3, 5 and 8). The monoclonal antibody G10/4 recognizes a conformational epitope in the VSPH7 VSP protein of the GS/M isolate. The monoclonal antibody 9B10 detects a non-conformational epitope in the VSP9B10 VSP protein of the WB isolate.

FIG. **3** shows a Western blot analysis detecting the presence of three *Giardia* VSPs (VSP9B10, VSP1267 and VSPH7) after trophozoite trypsinization. Trophozoites were treated with trypsin at 200 μg/ml and 2 mg/ml concentrations, or incubated in medium without trypsin (control). A control sample corresponding to a mouse anti-alkaline phosphatase antibody (α-mouse) in also shown.

FIG. **4** shows immunohistochemistry microphotographs of intestinal sections from gerbils infected with WB9B10 *Giardia* trophozoites (FIG. **4**A), non-infected gerbils (FIG. **4**B), and gerbils immunized with the entire repertoire of *Giardia* VSPs purified from transgenic trophozoites (FIG. **4**C).

FIG. **5**A shows the amino acid sequence of a recombinant *Giardia* VSP corresponding to the extracellular portion of VSP1267 plus a C-terminal His6 tag (boxed amino acids) (SEQ ID NO:1). The N-terminal signal peptide is underlined.

FIG. **5**B shows a Western blot detecting the recombinant VSP1267 using an anti-His6 monoclonal antibody.

FIG. **6** shows silver stained gels corresponding to insulins (NOVORAPID® and LANTUS®) preincubated with different concentrations of trypsin and pancreatine (a mixture of pancreatic juice components). The lane labeled MW corresponds to a molecular weight ladder. The "ins" and "trypsin" lanes are control lanes containing insulin and trypsin, respectively.

FIG. **7** shows blood glucose levels in female Balb/c mice, 7 weeks-old, which were left without food intake for 2 hours and then received the indicated doses of insulin. FIG. **7**A shows blood glucose levels after LANTUS® administration; whereas FIG. **7**B shows blood glucose levels after NOVORAPID® administration. The insulins were administered orally at 1 IU, 5 IU, and 50 IU doses. PBS and a subcutaneous administration of insulin at 1-5 IU were used as controls. 1 IU seemed to be a suboptimal dose for both insulins (circled).

FIG. **8** shows blood glucose levels in female Balb/c mice, 7 weeks-old, which were left without food intake for 2 hours and then received the indicated doses of insulin, alone or combined with a VSP carrier. FIG. **8**A shows blood glucose levels after LANTUS® administration; whereas FIG. **8**B shows blood glucose levels after NOVORAPID® administration. The insulins were administered at the suboptimal dose identified in FIG. **7** (1 IU) in three different formulations (i) insulin administered alone, (ii) insulin combined with VSP at a 1:1 molecule to molecule ratio, and (iii) insulin combined with VSP at a 1:3 molecule to molecule ratio. PBS and a subcutaneous administration of insulin at 1-5 IU were used as controls. The combination of 1 IU of insulin with a VSP carrier enhanced insulin's biological action, at a 1:1 insulin to VSP carrier ratio for LANTUS® and at a 1:3 insulin to VSP carrier ratio for NOVORAPID® (circled).

FIG. **9** shows the specificity of the anti-hGH monoclonal antibody (α HCB) by Western blot. Two dilutions of the monoclonal αHCB (1/3000 and 1/2000) were used to detect hGH (HCB: human growth hormone produced in transgenic bovines). A control containing an anti-alkaline phosphatase antibody (αMouse-AP1) is also shown.

FIG. **10**A shows the effect of pH on the stability of hGH. The top image is a Western blot showing the pH-mediated degradation of hGH incubated in medium at pH 1.6, 2.0, 3.8, 5.0, 5.8, 7.0, 8.0, 9.0, 10.0, and 11.0. The presence of hGH was determined using an anti-hGH monoclonal antibody. The bottom image shows the silver staining detection of hGH. Low pHs, similar to those found in the GIT, caused degradation of the protein (circles), while at higher pHs the hGH remained unaltered as compared with the control. Each lane contained 10 μg of hGH.

FIG. **10**B shows the effect of combining a VSP carrier with hGH on the hGH denaturation at low pH. The Western blots correspond to pairs of samples in which hGH samples without a VSP carrier or with a VSP carrier at a 1:3 hGH to VSP carrier ratio were subjected to the same pH conditions (pH 1.4, 1.96, 3.8, 4.91, 5.9, 7.01, 7.95, 8.51, 9.61, and 11.17). The hGH:VSP carrier sample at pH 3.8 was lost during processing. The presence of hGH was determined using an anti-hGH monoclonal antibody.

FIG. **11**A shows the effect of trypsin on the stability of hGH. The top image is a Western blot staining using an anti-hGH monoclonal antibody. The bottom image corresponds to silver staining. Trypsin completely proteolyzed hGH as indicated by the circled areas.

FIG. **11**B shows that combining a VSP carrier with hGH at a 1:3 hGH to VSP ratio protects hGH from trypsin degradation up to 150 μg/ml trypsin. The Western blots correspond to pairs of samples in which hGH samples without a VSP carrier, or with a VSP carrier at a 1:3 hGH to VSP carrier ratio were subjected to the same trypsin concentrations (50, 100, 150, 300 and 500 μg/ml). The presence of hGH was determined using an anti-hGH monoclonal antibody.

FIG. **12**A shows in its main panel the serum levels of hGH in mice after oral administration of the specified hGH doses (50, 100, 200, 400 and 800 μg). The inset shows the serum levels of hGH in mice after subcutaneous administration of a 12.5 μg dose of hGH.

FIG. **12**B shows serum levels of hGH in mice after oral administration of hGH alone or in combination with a VSP carrier at a 1:3 hGH to VSP carrier ratio.

FIG. **13**A shows the effect of trypsin on the stability of glucagon. The top panel of the Dot blot shows Trypsin proteolyzed glucagon. The bottom panel shows that combining a VSP carrier with glucagon at a 1:3 glucagon to VSP ratio protects glucagon from trypsin degradation up to 1:2 (protein:protease) ratio.

FIG. **13**B shows the effect on blood glucose levels resulting from oral administration of glucagon alone or combined with VSP to BALC/c mice.

DETAILED DESCRIPTION

Oral delivery of bioactive peptides such as insulin, glucagon, or growth hormone, which generally are administered via injection, offers considerable benefits in terms of decreased number of injections, improved compliance, and reduced incidence of side effects. However, successful oral delivery of therapeutic agents, e.g., bioactive peptides such as insulin, glucagon, or human growth hormone (hGH), involves overcoming the barriers of enzymatic degradation, achieving epithelial permeability, and taking steps to conserve bioactivity during the formulation process. To address this problem, we provide an oral delivery system in which bioactive peptides, e.g., insulin, glucagon, or hGH, are combined with, but not covalently combined via peptide bonds, with a VSP carrier to protect the bioactive peptides from degradation in the gastrointestinal track (GIT) and to promote its systemic biological action.

Accordingly, the present disclosure is directed to therapeutic compositions comprising VSP carriers (e.g., *Giardia* VSPs, VSP-like proteins, fragments, variants, or derivatives thereof) comprising at least one CXXC (SEQ ID NO:589) motif, wherein C represents a cysteine amino acid and X represents any amino acid, which can be combined and bind to therapeutic agents and function as carriers for drug delivery. The disclosure relates in particular to compositions comprising VSP carriers, e.g., polypeptides derived from the extracellular domain of *Giardia* VSP, which are resistant to proteases and different pHs, and which are able to attach to epithelial cells in the GIT. In some embodiments, such VSP carriers are used to form Virus-Like-Particles (VLPs) suitable to be administered orally.

The combination of therapeutic agents with VSP carriers for oral or mucosal administration confers to such therapeutic agents increased resistance to pH-induced degradation and enzymatic degradation, as well as increasing the binding of such therapeutic agents to the gastrointestinal epithelium. In some specific aspects, the therapeutics agents are bioactive peptides such as insulins, glucagon, or human growth hormone.

DEFINITIONS

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein.

Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A," (alone) and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or embodiments of the invention, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, are referred to by their commonly accepted single-letter codes.

As used herein, the terms "*Giardia*" or "*Giardia* parasite" refer to a genus of anaerobic flagellated protozoan parasites of the phylum Metamonada that colonize and reproduce in the small intestines of several vertebrates, causing giardiasis. World-wide, giardiasis is common among people with poor fecal-oral hygiene, and major modes of transmission include contaminated water supplies or sexual activity. Flagellated *Giardia* trophozoites attach to epithelial cells of the small intestine (i.e., the surface of the intestinal mucosa), where they can cause disease without triggering a pronounced inflammatory response (Rivero et al., Nat. Med. 16(5):551-7 (2010)). There are no known virulence factors or toxins, and variable expression of surface proteins allows evasion of

host immune responses and adaptation to different host environments (Rivero et al., Nat. Med. 16(5):551-7 (2010)). Their life cycle alternates between an actively swimming trophozoite and an infective, resistant cyst. The *Giardia* parasite infects humans, but is also one of the most common parasites infecting cats, dogs and birds. Mammalian hosts also include cows, beavers, deer, and sheep.

The term "*Giardia*" encompasses different species, including *Giardia lamblia* and *Giardia muris*. As used herein, the term "*Giardia lamblia*" (also called *Giardia* intestinalis or *Giardia duodenalis*) refers to one of the most common intestinal parasites of humans. *Giardia lamblia* is the most prevalent parasitic protist in the United States, where its incidence may be as high as 0.7% (Hlaysa et al., MMWR Surveill. Summ. 54:9-16 (2005)).

As used herein, the terms "variable surface protein," "VSP protein," or "VSP" refer to polypeptides that cover the entire surface of the *Giardia* parasite and are the major antigens recognized by the host immune system. The term "VSP" as defined herein also includes homologs, e.g., orthologs and paralogs of "VSP" proteins from *Giardia*, VSP and VSP-like proteins found in other organisms, as well as fragments, variants, and derivatives thereof.

The term "homolog," used with respect to a VSP protein or VSP-encoding gene of a first family or species, refers to distinct VSP protein or VSP-encoding genes of a second family or species which are determined by functional, structural, or genomic analyses to be an VSP protein or VSP-encoding gene of the second family or species which corresponds to the original VSP protein or VSP-encoding gene of the first family or species. As used herein, the term "homolog" refers to any VSP protein or VSP-encoding gene that is related to a reference VSP protein or VSP-encoding gene by descent from a common ancestral DNA sequence. The term homolog includes both orthologs and paralogs.

The term "ortholog" refers to VSP homologs in different species that evolved from a common ancestral gene by speciation. Typically, orthologs retain the same or similar function despite differences in their primary structure (mutations).

The term "paralog" refers to VSP homologs in the same species that evolved by genetic duplication of a common ancestral gene. In many cases, paralogs exhibit related (but not always identical functions). To the extent that a particular species has evolved multiple related genes from an ancestral DNA sequence shared with another species, the term ortholog can encompass the term paralog.

Most often, homologs will have functional, structural, or genomic similarities. Techniques are known by which homologs of an enzyme or gene can readily be cloned using genetic probes and PCR. Identity of cloned sequences as homologs can be confirmed using functional assays and/or by genomic mapping of the genes.

VSP proteins are cysteine-rich proteins with multiple CXXC (SEQ ID NO:589) motifs (where X is any amino acid) that have several particular characteristics, including in some VSP the presence of CXC motifs, a *Giardia*-specific Zinc-finger motif, and GGCY (SEQ ID NO:590) motifs (Nash, Mol. Microbiol. 45:585-590 (2002); Adam et al., BMC Genomics 10:424 (2010)). More precisely, VSP proteins are type 1 integral membrane proteins that vary in size from 20 to 200 kDa; possess a variable amino-terminal cysteine-rich region (extracellular domain that represents the host/parasite interface and confers to the protein resistance to proteolytic digestion and low pH), and a conserved carboxy-terminal region that includes a hydrophobic transmembrane region and a short cytosolic tail comprising only 5 amino acids (CRGKA) (SEQ ID NO:591), which are not "seen" by the immune system. Only one VSP protein is expressed at any given time on the surface of each parasite (Nash. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 352:1369-1375 (1997)).

Within the context of the present invention, the terms "variable surface protein," "VSP protein," or "VSP" includes any variable surface protein of the complete repertoire of *Giardia* VSP proteins, notably *Giardia lamblia*. Actually, *Giardia* parasites encodes a repertoire of about 200 genes encoding VSPs for VSP assemblage A (see, e.g., Morrison et al., Science 317:1921-1926 (2010); Adam et al., BMC Genomics 10:424 (2010)), and two reports of Svard's group describing the VSP repertoire of isolates derived from VSP assemblages B and E (Jerlstrom-Hultqvist et al. BMC Genomics 11:543 (2010); Franzen et al. PLoS Pathog. 5(8):c1000560 (2009)). The extracellular domain of a VSP allows the parasite to survive the hostile environment of the upper small intestine. VSPs are very resistant to variable pHs (reactivity to a conformational epitope by a monoclonal antibody directed to a particular VSP remains unaltered between pH 2 and 12), and digestion by trypsin and several other proteases. In addition, VSPs remain attached to the enteric mucosa after the trophozoites have attached to it (Rivero et al., Nat. Med. 16(5):551-7 (2010)). A comprehensive list of VSP proteins can be found at www.ebi.ac.uk/interpro/IEntry?ac=IPR005127.

It must be further noted that polypeptides comprising at least one CXXC (SEQ ID NO:589) motif, wherein C represents a cysteine residue and X any amino acid residue, such as *Giardia* VSPs or VSP-like proteins of other microorganisms may also be generated in vitro by genetic manipulation and produced in heterologous systems. Therefore, chemically- or cell-produced polypeptides, including those with amino acid variations not found in the wild type parasites (for instance variants of *Giardia* VSPs) are encompassed. VSPs may thus be prepared by any well-known procedure in the art, such as solid phase synthesis, liquid phase synthesis or genetic engineering.

VSPs used in the therapeutic compositions of the invention can undergo chemical modifications. Chemical modifications can be aimed at obtaining VSPs with increased protection against enzymatic degradation in vivo, and/or increased capacity to cross membrane barriers, thus increasing their half-lives and maintaining or improving their biological activity. Any chemical modification known in the art can be employed according to the present invention to modify a VSP. Such chemical modifications include but are not limited to:

(a) modifications to the N-terminal and/or C-terminal ends of the VSP proteins such as e.g., N-terminal acylation (preferably acetylation) or desamination, or modification of the C-terminal carboxyl group into an amide or an alcohol group;

(b) modifications at the amide bond between two amino acids: acylation (preferably acetylation) or alkylation (preferably methylation) at the nitrogen atom or the alpha carbon of the amide bond linking two amino acids;

(c) modifications at the alpha carbon of the amide bond linking two amino acids such as, e.g., acylation (preferably acetylation) or alkylation (preferably methylation) at the alpha carbon of the amide bond linking two amino acids.

(d) chirality changes such as, e.g., replacement of one or more naturally occurring amino acids (L enantiomer) with the corresponding D-enantiomers;

(e) retro-inversions in which one or more naturally-occurring amino acids (L-enantiomer) are replaced with the corresponding D-enantiomers, together with an inversion of the amino acid chain (from the C-terminal end to the N-terminal end); and/or

(f) azapeptides wherein one or more alpha carbons are replaced with nitrogen atoms.

The terms "protein" and "polypeptide," (e.g., a VSP protein) are used interchangeably to refer to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). Peptides, dipeptides, tripeptides, or oligopeptides are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be isolated from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. A polypeptide can be generated in any manner, including by chemical synthesis.

The terms "protein" or "polypeptide" (e.g., a VSP protein) also include variants which would encompass any polypeptide comprising any natural or genetically engineered polypeptide having at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least 99% amino acid sequence identity with the sequence of the polypeptide. Variant polypeptides can be generated using genetic engineered, e.g., by insertion, substitution, deletion, or a combination thereof. Substitutions in a protein sequence of the invention can be conservative or non-conservative.

When the term "variant of a protein" applies, according to the present invention, to the *Giardia* VSP or VSP-like protein of other microorganisms, such variant should be able of retaining the ability to attach to cells, particularly to mucosal cells, more particularly to epithelial cells of the GIT and functioning as a therapeutic agent carrier. Variants can be naturally or non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions

As used here, when the term "fragment" applies to a VSP or VSP-like protein of other microorganism (e.g., in the phrases "a fragment of a VSP" or a "VSP or a fragment thereof") such fragment should encompass any polypeptide comprising at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, or 500 contiguous or discontinuous amino acids of the protein or polypeptide as defined herein, as well as any polypeptide. Such fragment should be capable of retaining the ability to attach to cells, particularly to mucosal cell, more particularly to epithelial cells of the GIT and functioning as a therapeutic agent carrier.

"Derivatives" of polypeptides or proteins of the invention are polypeptides or proteins which have been altered so as to exhibit additional features not found on the native polypeptide or protein, but still display the beneficial properties of the parent polypeptide or protein (e.g., resistance to proteolytic enzyme degradation or binding to gastrointestinal epithelial cells).

An "isolated" polypeptide, protein, or a fragment, variant, or derivative thereof refers to a polypeptide or protein that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide or protein can simply be removed from its native or natural environment. A "recombinant" polypeptide or protein refers to a polypeptide or protein produced via recombinant DNA technology. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

A "protein sequence" or "amino acid sequence" means a linear representation of the amino acid constituents in a polypeptide in an amino-terminal to carboxyl-terminal direction in which residues that neighbor each other in the representation are contiguous in the primary structure of the polypeptide.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In another embodiment, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

The term "percent sequence identity" between two polynucleotide or polypeptide sequences refers to the number of identical matched positions shared by the sequences over a comparison window, taking into account additions or deletions (i.e., gaps) that must be introduced for optimal alignment of the two sequences. A matched position is any position where an identical nucleotide or amino acid is presented in both the target and reference sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides or amino acids. Likewise, gaps presented in the reference sequence are not counted since target sequence nucleotides or amino acids are counted, not nucleotides or amino acids from the reference sequence.

The percentage of sequence identity is calculated by determining the number of positions at which the identical amino-acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. The comparison of sequences and determination of percent sequence identity between two sequences may be accomplished using readily available software both for online use and for download. Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is bl2seq, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino

acid sequences. Other suitable programs are, e.g., Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at www.ebi.ac.uk/Tools/psa.

Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. Sequence alignments can be derived from multiple sequence alignments. One suitable program to generate multiple sequence alignments is ClustalW2, available from www.clustal.org. Another suitable program is MUSCLE, available from www.drive5.com/muscle/. ClustalW2 and MUSCLE are alternatively available, e.g., from the EBI.

It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (e.g., crystallographic protein structures), functional data (e.g., location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, e.g., from the EBI. It will also be appreciated that the final alignment used to calculated percent sequence identity may be curated either automatically or manually.

The terms "heterologous moiety" mean that a polynucleotide, polypeptide, non-peptidic polymer or other moiety is derived from a distinct entity from that of the entity to which it is being compared. For instance, a heterologous polypeptide can be synthetic, or derived from a different species, different cell type of an individual, or the same or different type of cell of distinct individuals. In one aspect, a heterologous moiety can be a polypeptide fused to another polypeptide to produce a fusion polypeptide or protein. In another aspect, a heterologous moiety can be a non-polypeptide. In some embodiments, the VSP carrier comprises a heterologous moiety, e.g., a His6 tag for protein purification. In other embodiments, therapeutic agents that are combined with VSP carriers provided herein can be conjugated or fused (recombinantly, or using protein synthesis or chemical conjugation methods) to at least one heterologous moiety, e.g., polyethylene glycol (PEG), to improve a pharmacokinetic and/or pharmacodynamics property (e.g., in vivo half-life). Heterologous moieties capable of increasing the in vivo half-life of therapeutic agents are known in the art.

The term "increased" with respect to a functional characteristic of a therapeutic agent such as resistance to degradation caused by high or low pH, resistance to enzymatic degradation (e.g., proteolytic degradation), or binding to target cells (e.g., gastrointestinal epithelial cells) is used to indicate that the relevant functional characteristic is increased relative to that of a reference (for example the therapeutic agent administered in the absence of a VSP carrier), as determined under comparable conditions.

In some embodiments, the increase in the functional characteristic of the therapeutic agent (e.g., resistance to enzymatic degradation in the GIT) is, e.g., at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% higher relative to a reference (for example resistance to enzymatic degradation of the therapeutic agent, e.g., insulin, in the GIT in the absence of a VSP carrier), as determined under comparable conditions.

In some embodiments, the increase in the functional characteristic of the therapeutic agent (e.g., resistance to enzymatic degradation in the GIT) is, e.g., an at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90-fold, or at least about 100-fold increase relative to a reference (for example when compared to the resistance to enzymatic degradation of the therapeutic agent, e.g., insulin, in the GIT in the absence of a VSP carrier), as determined under comparable conditions.

The term "decreased" with respect to a functional characteristic of a therapeutic agent such as resistance to degradation caused by high or low pH, resistance to enzymatic degradation (e.g., proteolytic degradation), or binding to target cells (e.g., gastrointestinal epithelial cells) is used to indicate that the relevant functional characteristic is decreased relative to that of a reference (for example the therapeutic agent administered in the absence of a VSP carrier), as determined under comparable conditions.

In some embodiments, the decrease in the functional characteristic of the therapeutic agent (e.g., resistance to enzymatic degradation in the GIT) is, e.g., at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% lower relative to a reference (for example resistance to enzymatic degradation of the therapeutic agent, e.g., insulin, in the GIT in the presence of a VSP carrier), as determined under comparable conditions.

In some embodiments, the decrease in the functional characteristic of the therapeutic agent (e.g., resistance to enzymatic degradation in the GIT) is, e.g., at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90-fold, or at least about 100-fold lower relative to a reference (for example when compared to the resistance to enzymatic degradation of the therapeutic agent, e.g., insulin, in the GIT in the presence of a VSP carrier), as determined under comparable conditions.

The term "polynucleotide" or "nucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, e.g., messenger RNA (mRNA) or plasmid DNA (pDNA). In certain embodiments, a polynucleotide com-

prises a conventional phosphodiester bond or a non-conventional bond (e.g., an amide bond, such as found in peptide nucleic acids (PNA)).

The term "nucleic acid" refers to any one or more nucleic acid segments, e.g., DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a VSP polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) from other polynucleotides in a solution. Isolated RNA molecules include in vivo or in vitro RNA transcripts of polynucleotides of the present invention. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid can include regulatory elements such as promoters, enhancers, ribosome binding sites, or transcription termination signals.

As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide. Two or more coding regions of the present invention can be present in a single polynucleotide construct, e.g., on a single vector, or in separate polynucleotide constructs, e.g., on separate (different) vectors. It follows, then, that a single vector can contain just a single coding region, or comprise two or more coding regions, e.g., a single vector can separately encode a binding domain-A and a binding domain-B as described below. In addition, a vector, polynucleotide, or nucleic acid of the invention can encode heterologous coding regions, either fused or unfused to a nucleic acid encoding a binding domain of the invention. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous moiety (e.g., a His6 tag).

Certain proteins secreted by eukaryotic cells are associated with a secretory signal peptide which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that signal peptides are generally fused to the N-terminus of the polypeptide, and are cleaved from the complete or "full-length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, a native signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous signal peptide, e.g., a human tissue plasminogen activator (TPA) or mouse β-glucuronidase signal peptide, or a functional derivative thereof, can be used.

As used herein, the term "host cell" refers to a cell or a population of cells harboring or capable of harboring a recombinant nucleic acid. Host cells can be a prokaryotic cells (e.g., *E. coli*), or alternatively, the host cells can be eukaryotic, for example, fungal cells (e.g., yeast cells such as *Saccharomyces cerivisiae, Pichia pastoris*, or *Schizosaccharomyces pombe*), and various animal cells, such as insect cells (e.g., Sf-9) or mammalian cells (e.g., HEK293F, CHO, COS-7, NIH-3T3).

The term "therapeutic agent" refers to any therapeutically active substance that is delivered to a subject, e.g., orally, to produce a desired beneficial effect such as preventing, inhibiting, or arresting the symptoms and/or progression of a disease or condition. In some embodiments, a therapeutic agent can be preformulated, e.g., as a microcapsule, microsphere, microbubble, liposome, nisome, emulsion, dispersion, etc., before it is combined with the VSP carrier. Also included in the definition of therapeutic agent are diagnostically active agents and imaging agents such as dyes or fluorescent markers.

As used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of U.S. or E.U. or other government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in humans. Hence, the term "pharmaceutically acceptable" refers to those properties and/or substances that are acceptable to a patient (e.g., a human patient) from a toxicological and/or safety point of view.

The terms "pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to excipients and carriers used in pharmaceutical compositions which do not have a significant detrimental impact on the treated host and which retain the therapeutic properties of the therapeutic agent with which it they are administered. One exemplary physiologically acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and are described, for example, in Remington's Pharmaceutical Sciences, (18^{t5} edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, Pa., incorporated herein by reference.

The phrase "effective amount" as used herein refers to that amount of a therapeutic composition of the invention, comprising a combination of a therapeutic agent with a VSP carrier, or a pharmaceutical composition comprising such therapeutic composition which is effective for producing a desired effect, at a reasonable benefit/risk ratio applicable to any medical treatment. For example, an "effective amount" is an amount effective to reduce or lessen at least one symptom of the disease or disorder being treated or to reduce or delay onset of one or more clinical markers or symptoms associated with the disease or disorder, or to modify or reverse the disease process.

The terms "treat" or "treatment" as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder in a subject, such as the progression of an hormone deficiency-related disease or condition. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable.

The term "treatment" also means prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

The term "administering," as used herein, means to give a therapeutic composition of the invention comprising a therapeutic agent combined with a VSP carrier, or pharmaceutical composition comprising the therapeutic composition of the invention, to a subject (e.g., human subject) in need thereof via a pharmaceutically acceptable route of administration. In some embodiments, the route of administration is oral or mucosal. In other embodiments, the route of administration is selected from subcutaneous, intramuscular, nasal, intravenous, and pulmonary administration. A VSP carrier can be administered as part of a pharmaceutical composition comprising at least one therapeutic agent and at least one pharmaceutically acceptable excipient.

The terms "subject" and "patient" are used interchangeably and refer to any individual, patient or animal, in particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, etc.

Introduction

This disclosure provides therapeutic compositions comprising a VSP carrier and a therapeutic agent. VSP carriers are polypeptides such as variant-specific surface proteins (VSPs) of the intestinal parasite *Giardia lamblia* and VSP-like proteins from other organisms which can bind to therapeutic agents, e.g., bioactive peptides, and effectively deliver such therapeutic agents by the oral or mucosal route. To determine whether VSPs, due to their resistance to degradation by acidic pH, resistance to proteolytic degradation, and adherence to the intestinal mucosa, can effectively be combined with therapeutic agents (e.g., bioactive peptide) and be used as carriers to transport therapeutic agents through the gastro intestinal tract (GIT), we have used three bioactive peptides, insulin, glucagon, and human growth hormone, as prototype therapeutic agents to be delivered by the oral route.

The results disclosed herein in the Examples section indicate that VSP carriers can be used to effectively deliver therapeutic agents orally, or by other delivery routes (e.g., mucosal administration) where proteolytic degradation and/or exposure to low pH could affect the integrity of the therapeutic agent.

The term "VSP carrier" as used herein refers to a VSP protein (e.g., a *Giardia* VSP, a VSP-like protein, a VSP or VSP-like protein fragment, a VSP or VSP-like protein variant, a VSP or VSP-like protein derivative, or a combination of two or more of said VSP polypeptides) which can bind to at least one therapeutic agent. In certain embodiments, the at least one therapeutic agent is not a vaccine immunogen.

The VSP carriers of the invention are not covalently bound to the therapeutic agents via peptidic bonds. Thus, the term "bound" and its grammatical variants (e.g., "bind," "binds," "binding," etc.) when applied to the interaction between a VSP carrier and a therapeutic agent refers to (i) covalent non-peptide binding (e.g., binding via a disulphide bond) or (ii) non-covalent binding, but not to peptide-bond formation between the VSP carrier and a therapeutic agent.

Non-limiting examples of non-covalent binding between a VSP carrier and a therapeutic agent include an ionic bond (e.g., cation-pi bond or salt bond), a metal bond, an hydrogen bond (e.g., dihydrogen bond, dihydrogen complex, low-barrier hydrogen bond, or symmetric hydrogen bond), van der Waals force, London dispersion force, a mechanical bond, a halogen bond, aurophilicity, intercalation, stacking, entropic force, or chemical polarity.

The term "bound" also refers to the enclosement, or partial enclosement of a therapeutic agent (e.g., a bioactive peptide such as insulin) by a molecular structure that comprises a VSP carrier. In some embodiments, the term "bound" refers to the interaction (covalent or non-covalent) of a VSP carrier with a macromolecular structure in which a therapeutic agent is enclosed. In this respect, the therapeutic agent can be enclosed or packaged, e.g., in a lipid bilayer, liposome, nanoparticle, nanotube, nanobubble, micelle, nanosphere, nanoshell, nanorod, chemical cage, nanohorn, quantum dot, nanocluster, microbubble, dendrimer, aquasome, lipopolyplex, nanoemulsion, or a combination thereof. The term "bound" also includes binding of a VSP carrier to a lipid bilayer or insertion of a VSP carrier into a lipid bilayer which either comprises the therapeutic agent (e.g., a liposoluble drug inserted in the bilayer's hydrophobic core) or encloses the therapeutic agent (e.g., the lipid bilayer is part of a liposome in which the therapeutic agent is packaged).

In some embodiments, the VSP carrier comprises a heterogous moiety genetically fused to a hydrophobic peptide that anchors the VSP carrier the bilayer. In other embodiments, a heterologous moiety that anchors the VSP carrier to the bilayer (e.g., a hydrophobic peptide or a lipid anchor) can be chemically conjugated to the VSP carrier. In some embodiments, the VSP carrier can be genetically fused or conjugated to a heterologous moiety by a linker. The term "linker" refers to a molecular entity that covalently links a VSP carrier and a heterologous moiety. The linker can comprises, for example, a thiol group, an alkyl group, a glycol group, or a peptide group. Linkers include cross-linking molecules. See, e.g., Int. Pat. Publ. No. WO 2004/009116 which is incorporated herein by reference in its entirety.

In some embodiments, the VSP carrier is bound to the therapeutic agent by forming a "virus-like particle" (VLP) which comprises the therapeutic agent (either on the surface of the VLP or encapsulated in the VLP). As used herein, the term "virus-like particle" or "VLP" refers to a structure resembling a virus particle that displays a *Giardia* VSP or a fragment thereof at its surface. A virus-like particle in accordance with the present invention is non-replicative since it lacks all or part of a viral genome, typically and preferably lacking all or part of the replicative and infectious components of a viral genome. The term "non-replicative" as used herein refers to being incapable of replicating the genome comprised or not in the VLP.

As used herein, the term "combine" (and grammatical variants such as "combined" or "combining") refers to the process of admixing two or more components (e.g., a VSP carrier and a therapeutic agent) such that contact between the components occur and such contact allows the binding of the two or more components.

In some specific embodiments, a therapeutic agent (e.g., a bioactive peptide such as IL-2) can be genetically fused to a VSP protein (see, e.g., preliminary experiments disclosed in the first paragraph of Example 9). In specific embodiments, the therapeutic agent is a bioactive peptide (e.g., insulin, glucagon, growth hormone) in a nanoparticle form which is chemically conjugated to a VSP. In some embodiments, a therapeutic agent (e.g., a bioactive peptide) is chemically conjugated to a VSP without any linker interposed between the therapeutic agent and the linker. In some embodiments, a therapeutic agent (e.g., a bioactive peptide) is chemically conjugated to a VSP with at least one linker interposed between the therapeutic agent and the VSP.

In some embodiments, a VSP carrier can be chemically conjugated with more than one therapeutic agent (e.g., more than one bioactive peptide). In some embodiments, two or more VSP carriers can be chemically conjugated with one therapeutic agent (e.g., a bioactive peptide). In other embodiments, two or more than two VSP carriers can be chemically conjugated with more than one therapeutic agent (e.g., more than one bioactive peptide). In some embodiments, a therapeutic agent is biologically active while genetically fused or chemically conjugated to a VSP carrier. In other embodiments, the therapeutic agent (e.g., a bioactive peptide) is inactive or only partially active while genetically fused or chemically conjugated to a VSP carrier, in which case, chemical and/or enzymatic cleavage can be required to release the active (or a more active) form of the therapeutic agent. Conversely, in some embodiments, the therapeutic agent (e.g., a bioactive peptide) is active while genetically fused or chemically conjugated to a VSP carrier, and chemical and/or enzymatic cleavage can be used to release the active form of the therapeutic agent from the VSP carrier in order to inactivate the therapeutic agent. Thus, a therapeutic agent (e.g., a bioactive peptide) could be protected by the VSP carrier while in a part of the GIT (e.g., the stomach), and degraded or inactivated after passage to a different part of the GIT by the different conditions prevalent in that portion of the GIT (e.g., different pH or specific enzymes).

In some embodiments, a therapeutic agent (e.g., a bioactive peptide) and a VSP carrier can be genetically fused or chemically conjugated by a peptide linker or other type of linker that can be cleaved (e.g., chemically or by a protease) such as the active form of the therapeutic peptide is released from the VSP carrier. In some embodiments, a therapeutic agent (e.g., a bioactive peptide) and a VSP carrier can be genetically fused or chemically conjugated by a peptide linker or other type of linker that can be cleaved (e.g., chemically or by a protease) such as the inactive form of the therapeutic agent is released from the VSP carrier.

In some embodiments, a therapeutic agent (e.g., a bioactive peptide) and a VSP carrier (bound, genetically fused, or chemically conjugated) can be bound, genetically fused, or chemically conjugated with another molecule to form a bivalent molecule (for example, both the VSP carrier and the therapeutic agent being genetically fused to the carboxy terminus of the beta chain of C4b-binding protein (C4PB)).

VSP Carriers from *Giardia* VSPs

In some embodiments, the VSP carrier comprises a VSP sequence chosen among the complete repertoire of VSPs which are encoded at the DNA level in the genome of the *Giardia* parasite. This repertoire is composed of about 200 homologous VSP-encoding genes (vsps), which varies in different *Giardia* isolates (see, Adam et al., BMC Genomics 11:424 (2010)). It should be further noted that variants of the *Giardia* VSPs, fragments, and derivatives can also be used as VSP carriers according to the invention. A representative, non-limiting list of proteins that can be used as VSP carriers is presented in TABLE 1.

TABLE 1

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
7	A0BQN4_PARTE	*Paramecium tetraurelia*
8	A0BR77_PARTE	*Paramecium tetraurelia*
9	A0D0W7_PARTE	*Paramecium tetraurelia*
10	A2E569_TRIVA	*Trichomonas vaginalis*
11	A2EKF7_TRIVA	*Trichomonas vaginalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
12	A6YSN6_GIAIN	*Giardia intestinalis*
13	A7SVH3_NEMVE	*Nematostella vectensis*
14	A8B1N7_GIAIC	*Giardia intestinalis*
15	A8B1Y1_GIAIC	*Giardia intestinalis*
16	A8B2D7_GIAIC	*Giardia intestinalis*
17	A8B2E6_GIAIC	*Giardia intestinalis*
18	A8B2P0_GIAIC	*Giardia intestinalis*
19	A8B2X6_GIAIC	*Giardia intestinalis*
20	A8B2Y3_GIAIC	*Giardia intestinalis*
21	A8B3P1_GIAIC	*Giardia intestinalis*
22	A8B3R3_GIAIC	*Giardia intestinalis*
23	A8B3V9_GIAIC	*Giardia intestinalis*
24	A8B497_GIAIC	*Giardia intestinalis*
25	A8B4C6_GIAIC	*Giardia intestinalis*
26	A8B4J7_GIAIC	*Giardia intestinalis*
27	A8B4K3_GIAIC	*Giardia intestinalis*
28	A8B4K7_GIAIC	*Giardia intestinalis*
29	A8B4P3_GIAIC	*Giardia intestinalis*
30	A8B4S6_GIAIC	*Giardia intestinalis*
31	A8B4Y0_GIAIC	*Giardia intestinalis*
32	A8B4Y3_GIAIC	*Giardia intestinalis*
33	A8B582_GIAIC	*Giardia intestinalis*
34	A8B5B4_GIAIC	*Giardia intestinalis*
35	A8B5M7_GIAIC	*Giardia intestinalis*
36	A8B5M8_GIAIC	*Giardia intestinalis*
37	A8B5P6_GIAIC	*Giardia intestinalis*
38	A8B5Q2_GIAIC	*Giardia intestinalis*
39	A8B5U7_GIAIC	*Giardia intestinalis*
40	A8B5U8_GIAIC	*Giardia intestinalis*
41	A8B6C8_GIAIC	*Giardia intestinalis*
42	A8B6F0_GIAIC	*Giardia intestinalis*
43	A8B6G2_GIAIC	*Giardia intestinalis*
44	A8B6J1_GIAIC	*Giardia intestinalis*
45	A8B6V3_GIAIC	*Giardia intestinalis*
46	A8B728_GIAIC	*Giardia intestinalis*
47	A8B7F5_GIAIC	*Giardia intestinalis*
48	A8B7F8_GIAIC	*Giardia intestinalis*
49	A8B7K8_GIAIC	*Giardia intestinalis*
50	A8B7T7_GIAIC	*Giardia intestinalis*
51	A8B838_GIAIC	*Giardia intestinalis*
52	A8B8E0_GIAIC	*Giardia intestinalis*
53	A8B8R6_GIAIC	*Giardia intestinalis*
54	A8B8Y3_GIAIC	*Giardia intestinalis*
55	A8B9G0_GIAIC	*Giardia intestinalis*
56	A8B9Q8_GIAIC	*Giardia intestinalis*
57	A8B9R0_GIAIC	*Giardia intestinalis*
58	A8BA10_GIAIC	*Giardia intestinalis*
59	A8BA87_GIAIC	*Giardia intestinalis*
60	A8BAG1_GIAIC	*Giardia intestinalis*
61	A8BAG4_GIAIC	*Giardia intestinalis*
62	A8BAI1_GIAIC	*Giardia intestinalis*
63	A8BAX4_GIAIC	*Giardia intestinalis*
64	A8BB06_GIAIC	*Giardia intestinalis*
65	A8BB29_GIAIC	*Giardia intestinalis*
66	A8BB81_GIAIC	*Giardia intestinalis*
67	A8BBE8_GIAIC	*Giardia intestinalis*
68	A8BBH5_GIAIC	*Giardia intestinalis*
69	A8BBP7_GIAIC	*Giardia intestinalis*
70	A8BBQ0_GIAIC	*Giardia intestinalis*
71	A8BBQ1_GIAIC	*Giardia intestinalis*
72	A8BBR6_GIAIC	*Giardia intestinalis*
73	A8BBX7_GIAIC	*Giardia intestinalis*
74	A8BC24_GIAIC	*Giardia intestinalis*
75	A8BC41_GIAIC	*Giardia intestinalis*
76	A8BCN1_GIAIC	*Giardia intestinalis*
77	A8BCU7_GIAIC	*Giardia intestinalis*
78	A8BCV1_GIAIC	*Giardia intestinalis*
79	A8BCV5_GIAIC	*Giardia intestinalis*
80	A8BCV8_GIAIC	*Giardia intestinalis*
81	A8BCW0_GIAIC	*Giardia intestinalis*
82	A8BCW5_GIAIC	*Giardia intestinalis*
83	A8BD73_GIAIC	*Giardia intestinalis*
84	A8BD76_GIAIC	*Giardia intestinalis*
85	A8BDC4_GIAIC	*Giardia intestinalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
86	A8BDH4_GIAIC	*Giardia intestinalis*
87	A8BDM0_GIAIC	*Giardia intestinalis*
88	A8BDP5_GIAIC	*Giardia intestinalis*
89	A8BEA2_GIAIC	*Giardia intestinalis*
90	A8BEA7_GIAIC	*Giardia intestinalis*
91	A8BEJ8_GIAIC	*Giardia intestinalis*
92	A8BEQ4_GIAIC	*Giardia intestinalis*
93	A8BET9_GIAIC	*Giardia intestinalis*
94	A8BEU2_GIAIC	*Giardia intestinalis*
95	A8BEV3_GIAIC	*Giardia intestinalis*
96	A8BFA0_GIAIC	*Giardia intestinalis*
97	A8BFC1_GIAIC	*Giardia intestinalis*
98	A8BFJ7_GIAIC	*Giardia intestinalis*
99	A8BFK4_GIAIC	*Giardia intestinalis*
100	A8BFY9_GIAIC	*Giardia intestinalis*
101	A8BFZ3_GIAIC	*Giardia intestinalis*
102	A8BG61_GIAIC	*Giardia intestinalis*
103	A8BGA3_GIAIC	*Giardia intestinalis*
104	A8BH77_GIAIC	*Giardia intestinalis*
105	A8BH92_GIAIC	*Giardia intestinalis*
106	A8BHI3_GIAIC	*Giardia intestinalis*
107	A8BHL4_GIAIC	*Giardia intestinalis*
108	A8BHY9_GIAIC	*Giardia intestinalis*
109	A8BJ28_GIAIC	*Giardia intestinalis*
110	A8BJM0_GIAIC	*Giardia intestinalis*
111	A8BJT8_GIAIC	*Giardia intestinalis*
112	A8BJU0_GIAIC	*Giardia intestinalis*
113	A8BJU2_GIAIC	*Giardia intestinalis*
114	A8BK37_GIAIC	*Giardia intestinalis*
115	A8BK84_GIAIC	*Giardia intestinalis*
116	A8BLI8_GIAIC	*Giardia intestinalis*
117	A8BLR0_GIAIC	*Giardia intestinalis*
118	A8BLZ5_GIAIC	*Giardia intestinalis*
119	A8BM49_GIAIC	*Giardia intestinalis*
120	A8BM52_GIAIC	*Giardia intestinalis*
121	A8BM73_GIAIC	*Giardia intestinalis*
122	A8BME9_GIAIC	*Giardia intestinalis*
123	A8BMY2_GIAIC	*Giardia intestinalis*
124	A8BN71_GIAIC	*Giardia intestinalis*
125	A8BNR0_GIAIC	*Giardia intestinalis*
126	A8BNR1_GIAIC	*Giardia intestinalis*
127	A8BNR4_GIAIC	*Giardia intestinalis*
128	A8BNZ3_GIAIC	*Giardia intestinalis*
129	A8BNZ4_GIAIC	*Giardia intestinalis*
130	A8BP21_GIAIC	*Giardia intestinalis*
131	A8BPL3_GIAIC	*Giardia intestinalis*
132	A8BPN5_GIAIC	*Giardia intestinalis*
133	A8BPN6_GIAIC	*Giardia intestinalis*
134	A8BPP1_GIAIC	*Giardia intestinalis*
135	A8BPP4_GIAIC	*Giardia intestinalis*
136	A8BPP7_GIAIC	*Giardia intestinalis*
137	A8BQ05_GIAIC	*Giardia intestinalis*
138	A8BQ57_GIAIC	*Giardia intestinalis*
139	A8BQ73_GIAIC	*Giardia intestinalis*
140	A8BQD0_GIAIC	*Giardia intestinalis*
141	A8BQD1_GIAIC	*Giardia intestinalis*
142	A8BQM2_GIAIC	*Giardia intestinalis*
143	A8BQM3_GIAIC	*Giardia intestinalis*
144	A8BQN8_GIAIC	*Giardia intestinalis*
145	A8BQX2_GIAIC	*Giardia intestinalis*
146	A8BQX8_GIAIC	*Giardia intestinalis*
147	A8BRF5_GIAIC	*Giardia intestinalis*
148	A8BRK4_GIAIC	*Giardia intestinalis*
149	A8BRR2_GIAIC	*Giardia intestinalis*
150	A8BRR9_GIAIC	*Giardia intestinalis*
151	A8BRS4_GIAIC	*Giardia intestinalis*
152	A8BRY1_GIAIC	*Giardia intestinalis*
153	A8BS16_GIAIC	*Giardia intestinalis*
154	A8BS49_GIAIC	*Giardia intestinalis*
155	A8BS56_GIAIC	*Giardia intestinalis*
156	A8BSC1_GIAIC	*Giardia intestinalis*
157	A8BSY8_GIAIC	*Giardia intestinalis*
158	A8BT57_GIAIC	*Giardia intestinalis*
159	A8BTL5_GIAIC	*Giardia intestinalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
160	A8BTQ7_GIAIC	*Giardia intestinalis*
161	A8BTR0_GIAIC	*Giardia intestinalis*
162	A8BTS2_GIAIC	*Giardia intestinalis*
163	A8BTV4_GIAIC	*Giardia intestinalis*
164	A8BTV5_GIAIC	*Giardia intestinalis*
165	A8BTZ8_GIAIC	*Giardia intestinalis*
166	A8BUT4_GIAIC	*Giardia intestinalis*
167	A8BUU6_GIAIC	*Giardia intestinalis*
168	A8BV34_GIAIC	*Giardia intestinalis*
169	A8BV90_GIAIC	*Giardia intestinalis*
170	A8BVA8_GIAIC	*Giardia intestinalis*
171	A8BVH9_GIAIC	*Giardia intestinalis*
172	A8BVM8_GIAIC	*Giardia intestinalis*
173	A8BVQ2_GIAIC	*Giardia intestinalis*
174	A8BVU8_GIAIC	*Giardia intestinalis*
175	A8BVV3_GIAIC	*Giardia intestinalis*
176	A8BVX4_GIAIC	*Giardia intestinalis*
177	A8BVZ5_GIAIC	*Giardia intestinalis*
178	A8BWB7_GIAIC	*Giardia intestinalis*
179	A8BWQ1_GIAIC	*Giardia intestinalis*
180	A8BXF2_GIAIC	*Giardia intestinalis*
181	A8BXV1_GIAIC	*Giardia intestinalis*
182	A8BY81_GIAIC	*Giardia intestinalis*
183	A8BY91_GIAIC	*Giardia intestinalis*
184	A8BYJ1_GIAIC	*Giardia intestinalis*
185	A8BYM1_GIAIC	*Giardia intestinalis*
186	A8BZ11_GIAIC	*Giardia intestinalis*
187	A8BZ19_GIAIC	*Giardia intestinalis*
188	A8BZM3_GIAIC	*Giardia intestinalis*
189	A8C008_GIAIC	*Giardia intestinalis*
190	A8C017_GIAIC	*Giardia intestinalis*
191	A8C020_GIAIC	*Giardia intestinalis*
192	A8C025_GIAIC	*Giardia intestinalis*
193	A8C028_GIAIC	*Giardia intestinalis*
194	A8C039_GIAIC	*Giardia intestinalis*
195	A8C046_GIAIC	*Giardia intestinalis*
196	A8C048_GIAIC	*Giardia intestinalis*
197	A8C081_GIAIC	*Giardia intestinalis*
198	A8C085_GIAIC	*Giardia intestinalis*
199	A8C088_GIAIC	*Giardia intestinalis*
200	A8C091_GIAIC	*Giardia intestinalis*
201	A8C094_GIAIC	*Giardia intestinalis*
202	A8C0A1_GIAIC	*Giardia intestinalis*
203	A8C0A4_GIAIC	*Giardia intestinalis*
204	A8C0A9_GIAIC	*Giardia intestinalis*
205	A8C0B7_GIAIC	*Giardia intestinalis*
206	A8C0C3_GIAIC	*Giardia intestinalis*
207	A8C0D1_GIAIC	*Giardia intestinalis*
208	A8C0E0_GIAIC	*Giardia intestinalis*
209	A8C0F1_GIAIC	*Giardia intestinalis*
210	A8C0F4_GIAIC	*Giardia intestinalis*
211	A8C0G2_GIAIC	*Giardia intestinalis*
212	A8C0H0_GIAIC	*Giardia intestinalis*
213	A8C0I3_GIAIC	*Giardia intestinalis*
214	A8C0J1_GIAIC	*Giardia intestinalis*
215	A8C0L4_GIAIC	*Giardia intestinalis*
216	A8C0N5_GIAIC	*Giardia intestinalis*
217	A8C0N9_GIAIC	*Giardia intestinalis*
218	A8C0R2_GIAIC	*Giardia intestinalis*
219	A8C0U5_GIAIC	*Giardia intestinalis*
220	A8C0V5_GIAIC	*Giardia intestinalis*
221	A8C0W0_GIAIC	*Giardia intestinalis*
222	A8C109_GIAIC	*Giardia intestinalis*
223	B0EAK2_ENTDS	*Entamoeba dispar*
224	B0EU80_ENTDS	*Entamoeba dispar*
225	B1N5Q9_ENTHI	*Entamoeba histolytica*
226	C4M6P7_ENTHI	*Entamoeba histolytica*
227	C4MAB2_ENTHI	*Entamoeba histolytica*
228	C6LMQ7_GIAIB	*Giardia intestinalis*
229	C6LMR0_GIAIB	*Giardia intestinalis*
230	C6LMY7_GIAIB	*Giardia intestinalis*
231	C6LN95_GIAIB	*Giardia intestinalis*
232	C6LND2_GIAIB	*Giardia intestinalis*
233	C6LNK7_GIAIB	*Giardia intestinalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
234	C6LNL8_GIAIB	*Giardia intestinalis*
235	C6LNP8_GIAIB	*Giardia intestinalis*
236	C6LNT8_GIAIB	*Giardia intestinalis*
237	C6LPA7_GIAIB	*Giardia intestinalis*
238	C6LPF5_GIAIB	*Giardia intestinalis*
239	C6LPK7_GIAIB	*Giardia intestinalis*
240	C6LPM5_GIAIB	*Giardia intestinalis*
241	C6LQL2_GIAIB	*Giardia intestinalis*
242	C6LR62_GIAIB	*Giardia intestinalis*
243	C6LR84_GIAIB	*Giardia intestinalis*
244	C6LRD7_GIAIB	*Giardia intestinalis*
245	C6LSQ1_GIAIB	*Giardia intestinalis*
246	C6LT09_GIAIB	*Giardia intestinalis*
247	C6LT91_GIAIB	*Giardia intestinalis*
248	C6LTI3_GIAIB	*Giardia intestinalis*
249	C6LTI4_GIAIB	*Giardia intestinalis*
250	C6LUA9_GIAIB	*Giardia intestinalis*
251	C6LUN4_GIAIB	*Giardia intestinalis*
252	C6LV02_GIAIB	*Giardia intestinalis*
253	C6LV68_GIAIB	*Giardia intestinalis*
254	C6LVB6_GIAIB	*Giardia intestinalis*
255	C6LVG2_GIAIB	*Giardia intestinalis*
256	C6LVI0_GIAIB	*Giardia intestinalis*
257	C6LVL2_GIAIB	*Giardia intestinalis*
258	C6LVZ6_GIAIB	*Giardia intestinalis*
259	C6LW79_GIAIB	*Giardia intestinalis*
260	C6LW95_GIAIB	*Giardia intestinalis*
261	C6LW96_GIAIB	*Giardia intestinalis*
262	C6LWD6_GIAIB	*Giardia intestinalis*
263	C6LWL1_GIAIB	*Giardia intestinalis*
264	C6LWM7_GIAIB	*Giardia intestinalis*
265	C6LWN2_GIAIB	*Giardia intestinalis*
266	C6LWN3_GIAIB	*Giardia intestinalis*
267	C6LWN4_GIAIB	*Giardia intestinalis*
268	C6LWQ8_GIAIB	*Giardia intestinalis*
269	C6LX19_GIAIB	*Giardia intestinalis*
270	C6LX20_GIAIB	*Giardia intestinalis*
271	C6LXE3_GIAIB	*Giardia intestinalis*
272	C6LXG6_GIAIB	*Giardia intestinalis*
273	C6LXK0_GIAIB	*Giardia intestinalis*
274	C6LYE5_GIAIB	*Giardia intestinalis*
275	C6M071_GIAIB	*Giardia intestinalis*
276	C6M0C1_GIAIB	*Giardia intestinalis*
277	D3KID0_GIAIC	*Giardia intestinalis*
278	D3KID3_GIAIC	*Giardia intestinalis*
279	E1EVI9_GIAIA	*Giardia intestinalis*
280	E1EVN5_GIAIA	*Giardia intestinalis*
281	E1EVS1_GIAIA	*Giardia intestinalis*
282	E1EVS6_GIAIA	*Giardia intestinalis*
283	E1EVZ6_GIAIA	*Giardia intestinalis*
284	E1EW20_GIAIA	*Giardia intestinalis*
285	E1EW38_GIAIA	*Giardia intestinalis*
286	E1EW69_GIAIA	*Giardia intestinalis*
287	E1EWA1_GIAIA	*Giardia intestinalis*
288	E1EWG3_GIAIA	*Giardia intestinalis*
289	E1EWG5_GIAIA	*Giardia intestinalis*
290	E1EWJ7_GIAIA	*Giardia intestinalis*
291	E1EWL5_GIAIA	*Giardia intestinalis*
292	E1EWV5_GIAIA	*Giardia intestinalis*
293	E1EX39_GIAIA	*Giardia intestinalis*
294	E1EX68_GIAIA	*Giardia intestinalis*
295	E1EX83_GIAIA	*Giardia intestinalis*
296	E1EX85_GIAIA	*Giardia intestinalis*
297	E1EX98_GIAIA	*Giardia intestinalis*
298	E1EXE5_GIAIA	*Giardia intestinalis*
299	E1EXF3_GIAIA	*Giardia intestinalis*
300	E1EXH8_GIAIA	*Giardia intestinalis*
301	E1EXH9_GIAIA	*Giardia intestinalis*
302	E1EXL8_GIAIA	*Giardia intestinalis*
303	E1EXQ8_GIAIA	*Giardia intestinalis*
304	E1EXV3_GIAIA	*Giardia intestinalis*
305	E1EXV4_GIAIA	*Giardia intestinalis*
306	E1EXY2_GIAIA	*Giardia intestinalis*
307	E1EYP5_GIAIA	*Giardia intestinalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
308	E1EYT9_GIAIA	*Giardia intestinalis*
309	E1EYU0_GIAIA	*Giardia intestinalis*
310	E1EZ23_GIAIA	*Giardia intestinalis*
311	E1EZ44_GIAIA	*Giardia intestinalis*
312	E1EZ63_GIAIA	*Giardia intestinalis*
313	E1EZD7_GIAIA	*Giardia intestinalis*
314	E1EZF7_GIAIA	*Giardia intestinalis*
315	E1EZI4_GIAIA	*Giardia intestinalis*
316	E1EZI5_GIAIA	*Giardia intestinalis*
317	E1EZR7_GIAIA	*Giardia intestinalis*
318	E1EZR8_GIAIA	*Giardia intestinalis*
319	E1EZZ8_GIAIA	*Giardia intestinalis*
320	E1F044_GIAIA	*Giardia intestinalis*
321	E1F046_GIAIA	*Giardia intestinalis*
322	E1F0C0_GIAIA	*Giardia intestinalis*
323	E1F0K6_GIAIA	*Giardia intestinalis*
324	E1F0M1_GIAIA	*Giardia intestinalis*
325	E1F0M8_GIAIA	*Giardia intestinalis*
326	E1F0M9_GIAIA	*Giardia intestinalis*
327	E1F0N0_GIAIA	*Giardia intestinalis*
328	E1F0N3_GIAIA	*Giardia intestinalis*
329	E1F0P7_GIAIA	*Giardia intestinalis*
330	E1F0T4_GIAIA	*Giardia intestinalis*
331	E1F129_GIAIA	*Giardia intestinalis*
332	E1F130_GIAIA	*Giardia intestinalis*
333	E1F1D1_GIAIA	*Giardia intestinalis*
334	E1F1F5_GIAIA	*Giardia intestinalis*
335	E1F1J9_GIAIA	*Giardia intestinalis*
336	E1F1K4_GIAIA	*Giardia intestinalis*
337	E1F1S0_GIAIA	*Giardia intestinalis*
338	E1F1U0_GIAIA	*Giardia intestinalis*
339	E1F1Y3_GIAIA	*Giardia intestinalis*
340	E1F1Y4_GIAIA	*Giardia intestinalis*
341	E1F1Z6_GIAIA	*Giardia intestinalis*
342	E1F213_GIAIA	*Giardia intestinalis*
343	E1F225_GIAIA	*Giardia intestinalis*
344	E1F249_GIAIA	*Giardia intestinalis*
345	E1F2E8_GIAIA	*Giardia intestinalis*
346	E1F2L4_GIAIA	*Giardia intestinalis*
347	E1F2Q5_GIAIA	*Giardia intestinalis*
348	E1F2S0_GIAIA	*Giardia intestinalis*
349	E1F2V4_GIAIA	*Giardia intestinalis*
350	E1F2Z8_GIAIA	*Giardia intestinalis*
351	E1F326_GIAIA	*Giardia intestinalis*
352	E1F373_GIAIA	*Giardia intestinalis*
353	E1F3A2_GIAIA	*Giardia intestinalis*
354	E1F3D7_GIAIA	*Giardia intestinalis*
355	E1F3H3_GIAIA	*Giardia intestinalis*
356	E1F3M4_GIAIA	*Giardia intestinalis*
357	E1F3S1_GIAIA	*Giardia intestinalis*
358	E1F3U6_GIAIA	*Giardia intestinalis*
359	E1F3X6_GIAIA	*Giardia intestinalis*
360	E1F3X7_GIAIA	*Giardia intestinalis*
361	E1F428_GIAIA	*Giardia intestinalis*
362	E1F447_GIAIA	*Giardia intestinalis*
363	E1F459_GIAIA	*Giardia intestinalis*
364	E1F477_GIAIA	*Giardia intestinalis*
365	E1F478_GIAIA	*Giardia intestinalis*
366	E1F4C2_GIAIA	*Giardia intestinalis*
367	E1F4C3_GIAIA	*Giardia intestinalis*
368	E1F4G3_GIAIA	*Giardia intestinalis*
369	E1F4G6_GIAIA	*Giardia intestinalis*
370	E1F4L3_GIAIA	*Giardia intestinalis*
371	E1F4L5_GIAIA	*Giardia intestinalis*
372	E1F4N7_GIAIA	*Giardia intestinalis*
373	E1F4N8_GIAIA	*Giardia intestinalis*
374	E1F4N9_GIAIA	*Giardia intestinalis*
375	E1F4X0_GIAIA	*Giardia intestinalis*
376	E1F562_GIAIA	*Giardia intestinalis*
377	E1F591_GIAIA	*Giardia intestinalis*
378	E1F598_GIAIA	*Giardia intestinalis*
379	E1F5C1_GIAIA	*Giardia intestinalis*
380	E1F5E4_GIAIA	*Giardia intestinalis*
381	E1F5I0_GIAIA	*Giardia intestinalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
382	E1F5N4_GIAIA	*Giardia intestinalis*
383	E1F5N5_GIAIA	*Giardia intestinalis*
384	E1F5Q7_GIAIA	*Giardia intestinalis*
385	E1F5Q8_GIAIA	*Giardia intestinalis*
386	E1F5R1_GIAIA	*Giardia intestinalis*
387	E1F5S2_GIAIA	*Giardia intestinalis*
388	E1F5W0_GIAIA	*Giardia intestinalis*
389	E1F5W1_GIAIA	*Giardia intestinalis*
390	E1F5X3_GIAIA	*Giardia intestinalis*
391	E1F5Y9_GIAIA	*Giardia intestinalis*
392	E1F5Z9_GIAIA	*Giardia intestinalis*
393	E1F623_GIAIA	*Giardia intestinalis*
394	E1F663_GIAIA	*Giardia intestinalis*
395	E1F667_GIAIA	*Giardia intestinalis*
396	E1F670_GIAIA	*Giardia intestinalis*
397	E1F685_GIAIA	*Giardia intestinalis*
398	E1F6C5_GIAIA	*Giardia intestinalis*
399	E1F6G2_GIAIA	*Giardia intestinalis*
400	E1F6H9_GIAIA	*Giardia intestinalis*
401	E1F6I0_GIAIA	*Giardia intestinalis*
402	E1F6L8_GIAIA	*Giardia intestinalis*
403	E1F6M3_GIAIA	*Giardia intestinalis*
404	E1F6Q9_GIAIA	*Giardia intestinalis*
405	E1F6T5_GIAIA	*Giardia intestinalis*
406	E1F6V8_GIAIA	*Giardia intestinalis*
407	E1F727_GIAIA	*Giardia intestinalis*
408	E1F734_GIAIA	*Giardia intestinalis*
409	E1F771_GIAIA	*Giardia intestinalis*
410	E1F772_GIAIA	*Giardia intestinalis*
411	E1F797_GIAIA	*Giardia intestinalis*
412	E1F7B5_GIAIA	*Giardia intestinalis*
413	E1F7B6_GIAIA	*Giardia intestinalis*
414	E1F7D7_GIAIA	*Giardia intestinalis*
415	E1F7F9_GIAIA	*Giardia intestinalis*
416	E1F7P6_GIAIA	*Giardia intestinalis*
417	E1F7U6_GIAIA	*Giardia intestinalis*
418	E1F7W8_GIAIA	*Giardia intestinalis*
419	E1F7X0_GIAIA	*Giardia intestinalis*
420	E1F7X8_GIAIA	*Giardia intestinalis*
421	E1F7Z9_GIAIA	*Giardia intestinalis*
422	E1F856_GIAIA	*Giardia intestinalis*
423	E1F891_GIAIA	*Giardia intestinalis*
424	E1F8E1_GIAIA	*Giardia intestinalis*
425	E1F8F3_GIAIA	*Giardia intestinalis*
426	E1F8K1_GIAIA	*Giardia intestinalis*
427	E1F8M3_GIAIA	*Giardia intestinalis*
428	E1F8N7_GIAIA	*Giardia intestinalis*
429	E1F8N8_GIAIA	*Giardia intestinalis*
430	E1F8P0_GIAIA	*Giardia intestinalis*
431	E1F8P4_GIAIA	*Giardia intestinalis*
432	E1F8Q3_GIAIA	*Giardia intestinalis*
433	E1F8Q5_GIAIA	*Giardia intestinalis*
434	E1F8Q9_GIAIA	*Giardia intestinalis*
435	E1F8S0_GIAIA	*Giardia intestinalis*
436	E1F8S1_GIAIA	*Giardia intestinalis*
437	E1F8U6_GIAIA	*Giardia intestinalis*
438	E1F8U7_GIAIA	*Giardia intestinalis*
439	E1F8V9_GIAIA	*Giardia intestinalis*
440	E1F8W0_GIAIA	*Giardia intestinalis*
441	E1F8W3_GIAIA	*Giardia intestinalis*
442	E1F8Y1_GIAIA	*Giardia intestinalis*
443	E1F8Z1_GIAIA	*Giardia intestinalis*
444	E1F917_GIAIA	*Giardia intestinalis*
445	E1F930_GIAIA	*Giardia intestinalis*
446	E1F954_GIAIA	*Giardia intestinalis*
447	E1F987_GIAIA	*Giardia intestinalis*
448	E1F9D3_GIAIA	*Giardia intestinalis*
449	E1F9D5_GIAIA	*Giardia intestinalis*
450	E1F9E4_GIAIA	*Giardia intestinalis*
451	E1F9F7_GIAIA	*Giardia intestinalis*
452	E1F9H3_GIAIA	*Giardia intestinalis*
453	E1F9I2_GIAIA	*Giardia intestinalis*
454	E1F9I5_GIAIA	*Giardia intestinalis*
455	E1F9J0_GIAIA	*Giardia intestinalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
456	E1F9J2_GIAIA	*Giardia intestinalis*
457	E1F9L1_GIAIA	*Giardia intestinalis*
458	E1F9M5_GIAIA	*Giardia intestinalis*
459	E1F9M9_GIAIA	*Giardia intestinalis*
460	E1F9N1_GIAIA	*Giardia intestinalis*
461	E1F9N2_GIAIA	*Giardia intestinalis*
462	E1F9Q7_GIAIA	*Giardia intestinalis*
463	E1F9R4_GIAIA	*Giardia intestinalis*
464	E1F9R6_GIAIA	*Giardia intestinalis*
465	E1F9U1_GIAIA	*Giardia intestinalis*
466	E2RTM9_GIAIN	*Giardia intestinalis*
467	E2RTN8_GIAIC	*Giardia intestinalis*
468	E2RTU6_GIAIC	*Giardia intestinalis*
469	E2RTV3_GIAIC	*Giardia intestinalis*
470	E2RTX4_GIAIC	*Giardia intestinalis*
471	E2RU01_GIAIC	*Giardia intestinalis*
472	E2RU12_GIAIC	*Giardia intestinalis*
473	E2RU28_GIAIC	*Giardia intestinalis*
474	E2RU34_GIAIC	*Giardia intestinalis*
475	E2RU43_GIAIC	*Giardia intestinalis*
476	E2RU54_GIAIC	*Giardia intestinalis*
477	E5EZ44_GIAIN	*Giardia intestinalis*
478	E5EZ45_GIAIN	*Giardia intestinalis*
479	E5EZ46_GIAIN	*Giardia intestinalis*
480	E5EZ47_GIAIN	*Giardia intestinalis*
481	G0QQQ1_ICHMG	*Ichthyophthirius multifiliis*
482	G0QTU6_ICHMG	*Ichthyophthirius multifiliis*
483	O97443_GIAIN	*Giardia intestinalis*
484	O97444_GIAIN	*Giardia intestinalis*
485	O97448_GIAIN	*Giardia intestinalis*
486	O97450_GIAIN	*Giardia intestinalis*
487	TSA4_GIAIN	*Giardia intestinalis*
488	VS41_GIAIN	*Giardia intestinalis*
489	TS11_GIAIN	*Giardia intestinalis*
490	Q07317_GIAIN	*Giardia intestinalis*
491	Q0R0E0_GIAIN	*Giardia intestinalis*
492	Q22M55_TETTS	*Tetrahymena thermophila*
493	Q234X6_TETTS	*Tetrahymena thermophila*
494	Q24959_GIAIN	*Giardia intestinalis*
495	Q24960_GIAIN	*Giardia intestinalis*
496	Q24962_GIAIN	*Giardia intestinalis*
497	Q24970_GIAIN	*Giardia intestinalis*
498	Q24971_GIAIN	*Giardia intestinalis*
499	Q24977_GIAIN	*Giardia intestinalis*
500	Q24986_GIAIN	*Giardia intestinalis*
501	Q24987_GIAIN	*Giardia intestinalis*
502	Q24988_GIAIN	*Giardia intestinalis*
503	Q24990_GIAIN	*Giardia intestinalis*
504	Q24992_GIAIN	*Giardia intestinalis*
505	Q38QK0_GIAIN	*Giardia intestinalis*
506	Q49L26_GIAMU	*Giardia muris*
507	Q49L27_GIAMU	*Giardia muris*
508	Q49L28_GIAMU	*Giardia muris*
509	Q49L29_GIAMU	*Giardia muris*
510	Q49L30_GIAMU	*Giardia muris*
511	Q49L31_GIAMU	*Giardia muris*
512	Q4RPQ0_TETNG	*Tetraodon nigroviridis*
513	Q7JNB5_GIAIN	*Giardia intestinalis*
514	Q7M3R4_GIAIN	*Giardia intestinalis*
515	Q8I0M3_GIAIN	*Giardia intestinalis*
516	Q8I0P4_GIAIN	*Giardia intestinalis*
517	Q8I8V1_GIAIN	*Giardia intestinalis*
518	Q8I8V2_GIAIN	*Giardia intestinalis*
519	Q8I8V3_GIAIN	*Giardia intestinalis*
520	Q8I8V4_GIAIN	*Giardia intestinalis*
521	Q8I8V5_GIAIN	*Giardia intestinalis*
522	Q8I8V6_GIAIN	*Giardia intestinalis*
523	Q8I8V7_GIAIN	*Giardia intestinalis*
524	Q8I8V8_GIAIN	*Giardia intestinalis*
525	Q8I8V9_GIAIN	*Giardia intestinalis*
526	Q8I8W0_GIAIN	*Giardia intestinalis*
527	Q8I8W1_GIAIN	*Giardia intestinalis*
528	Q8I8W2_GIAIN	*Giardia intestinalis*
529	Q8I8W3_GIAIN	*Giardia intestinalis*

TABLE 1-continued

Exemplary list of VSP and VSP-like proteins that can be used as VSP carriers.

SEQ ID NO	UNIPROT IDENTIFIER	SPECIES
530	Q8I8W4_GIAIN	*Giardia intestinalis*
531	Q8I8W6_GIAIN	*Giardia intestinalis*
532	Q8MPM6_GIAIN	*Giardia intestinalis*
533	Q95PT9_GIAIN	*Giardia intestinalis*
534	Q95WU1_GIAIN	*Giardia intestinalis*
535	Q967R8_GIAIN	*Giardia intestinalis*
536	Q967R9_GIAIN	*Giardia intestinalis*
537	Q9BH65_GIAIN	*Giardia intestinalis*
538	Q9BIJ8_GIAIN	*Giardia intestinalis*
539	Q9BIJ9_GIAIN	*Giardia intestinalis*
540	Q9BIK0_GIAIN	*Giardia intestinalis*
541	Q9BIK1_GIAIN	*Giardia intestinalis*
542	Q9BIK2_GIAIN	*Giardia intestinalis*
543	Q9BIK3_GIAIN	*Giardia intestinalis*
544	Q9BIK4_GIAIN	*Giardia intestinalis*
545	Q9BIK5_GIAIN	*Giardia intestinalis*
546	Q9BIK6_GIAIN	*Giardia intestinalis*
547	Q9BIK7_GIAIN	*Giardia intestinalis*
548	Q9BIK8_GIAIN	*Giardia intestinalis*
549	Q9BIK9_GIAIN	*Giardia intestinalis*
550	Q9BIL0_GIAIN	*Giardia intestinalis*
551	Q9BIL1_GIAIN	*Giardia intestinalis*
552	Q9BIL2_GIAIN	*Giardia intestinalis*
553	Q9BIL3_GIAIN	*Giardia intestinalis*
554	Q9BIL4_GIAIN	*Giardia intestinalis*
555	Q9BIL5_GIAIN	*Giardia intestinalis*
556	Q9BIL6_GIAIN	*Giardia intestinalis*
557	Q9BIL7_GIAIN	*Giardia intestinalis*
558	Q9BIL8_GIAIN	*Giardia intestinalis*
559	Q9BIL9_GIAIN	*Giardia intestinalis*
560	Q9BIM0_GIAIN	*Giardia intestinalis*
561	Q9BIM1_GIAIN	*Giardia intestinalis*
562	Q9BIM2_GIAIN	*Giardia intestinalis*
563	Q9BIM3_GIAIN	*Giardia intestinalis*
564	Q9GQ40_GIAIN	*Giardia intestinalis*
565	Q9GQ41_GIAIN	*Giardia intestinalis*
566	Q9GQ42_GIAIN	*Giardia intestinalis*
567	Q9GQ43_GIAIN	*Giardia intestinalis*
568	Q9GQ44_GIAIN	*Giardia intestinalis*
569	Q9GQ45_GIAIN	*Giardia intestinalis*
570	Q9GQ46_GIAIN	*Giardia intestinalis*
571	Q9GQ47_GIAIN	*Giardia intestinalis*
572	Q9GS24_GIAIN	*Giardia intestinalis*
573	Q9GSP6_GIAIN	*Giardia intestinalis*
574	Q9NGL3_GIAIN	*Giardia intestinalis*
575	Q9NGZ3_GIAIN	*Giardia intestinalis*
576	Q9NGZ6_GIAIN	*Giardia intestinalis*
577	Q9NGZ7_GIAIN	*Giardia intestinalis*
578	Q9NH87_GIAIN	*Giardia intestinalis*
579	Q9U013_GIAIN	*Giardia intestinalis*
580	Q9U018_GIAIN	*Giardia intestinalis*
581	Q9U019_GIAIN	*Giardia intestinalis*
582	Q9U021_GIAIN	*Giardia intestinalis*
583	Q9U048_GIAIN	*Giardia intestinalis*
584	Q9U063_GIAIN	*Giardia intestinalis*
585	Q9U064_GIAIN	*Giardia intestinalis*
586	Q9XTJ7_GIAIN	*Giardia intestinalis*
587	Q9XTK3_GIAIN	*Giardia intestinalis*
588	Q9XY90_GIAIN	*Giardia intestinalis*

TABLE 1 includes the sequences of 585 VSP and VSP-like proteins comprising Interpro *Giardia* variant-specific surface protein motif IPR005127 (sequence list published online at embl-ebi.org/interpro/IEntry?ac=IPR005127 and publicly available on Jun. 12, 2012). Other VSP and VSP-like proteins that can be used as VSP carriers according to the present disclosure are the 1,079 protein sequences comprising the *Giardia* variant-specific surface protein motif PF03302 available at Version 26.0 of the Pfam database (sequence list published online at pfam.sanger.ac.uk/family/PF03302 and publicly available on Jun. 12, 2012). The lists of protein sequences published in the above cited databases and publicly available on the above disclosed dates are herein incorporated by reference in their entireties.

As above-mentioned, the *Giardia* VSPs and more particularly the extracellular domain of the *Giardia* VSPs comprise multiple CXXC (SEQ ID NO:589) motifs, preferably multiple CXXC (SEQ ID NO:589) motifs separated by several amino acids, from 3 to 20 amino acids and more particularly from 5 to 8 amino acids (as observed by multiple sequence alignments). Thus, in some embodiments, the VSP carrier is a fragment, analog or derivative of a VSP or VSP-like protein from *Giardia*, wherein the VSP carrier comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 CXXC (SEQ ID NO:589) motifs. In some embodiments, the VSP carrier comprises at least about 40, at least about 50, at least about 60, at least about 70, at least 80, about 90 or at least about 100 CXXC (SEQ ID NO:589) motifs from a *Giardia* VSP.

In a particular embodiment, the *Giardia* parasite is *Giardia lamblia*. In one embodiment, the *Giardia* VSP can be without limitation VSP9B10 (Uniprot:Q9GS24), VSP1267 (Uniprot:Q07317), VSPA6 (Uniprot:Q24970), VSPS1 (Uniprot:Q8I0P4), VSPS2 (Uniprot:Q8I8W6), VSPS3 (Uniprot: Q8I0M3), VSPS4 (Uniprot: E2RTM9), VSPS5 (Uniprot: Q8I8W4), VSPS6 (Uniprot:Q8I8W3), VSPS7 (Uniprot: Q8I8W2), VSPS8 (Uniprot:E2RTU6), VSPAS1 (Uniprot: Q8I0M3), VSPAS2 (Uniprot:818W0), VSPAS3 (Uniprot: Q8I8V9), VSPAS4 (Uniprot:Q8I0P4), VSPAS5 (Uniprot: Q8I8V8), VSPAS6 (Uniprot:Q8I8V7), VSPAS7 (Uniprot: Q8I8V6), VSPAS8 (Uniprot:Q8I8V5), VSPAS9 (Uniprot: Q8I8V4), VSPAS10 (Uniprot:Q8I8V3), VSPAS11 (Uniprot: Q8I8V2), VSPAS12 (Uniprot:E2RU01) or VSPH7 (Uniprot: □24992) of *Giardia lamblia*, or fragments, variants, or derivatives thereof.

In one embodiment, a VSP carrier comprises the extracellular domain of a *Giardia* VSP, or a fragment, variant or derivative thereof (since said extracellular domain is the amino-terminal cysteine rich region comprising multiple CXXC (SEQ ID NO:589) motifs of the *Giardia* VSP protein).

The extracellular domain of a *Giardia* VSP is the domain resistant to the pH, temperature and proteolytic digestion. Accordingly, in another embodiment, the VSP carrier according to the invention comprises only the extracellular domain of a *Giardia* VSP, or a fragment, variant, or derivative thereof. In a particular embodiment, the VSP carrier comprises the extracellular sequence of VSP1267 (SEQ ID NO:2), comprising the N-terminal signal peptide. The transmembrane region and the cytoplasmic tail of a *Giardia* VSP are thus eliminated. It should be noted that the peptide signal can also be removed from the VSP extracellular domain.

A list of VSP and VSP-like proteins suitable to generate VSP carriers, including their sequences, Uniprot Accession Numbers, and Uniprot Entry Names is included at the end of this specification. The correspondence between Uniprot Accession Nos. and Entry Names and VSP protein number can be determined from the Uniprot entry at www.uniprot.org. E.g., Uniprot Accession No, Q07317 and its Entry Name Q07317_GIAIN would correspond to VSP1267.

The term "VSP1267 carrier" refers to a recombinantly produced fragment of the *Giardia* VSP protein VSP1267 without the transmembrane domain and cytosolic tail, but with the signal peptide intact and further comprising a C-terminal His6 tag (SEQ ID NO:1, shown in FIG. **5**A). In some embodiments, a VSP carrier is a fragment, variant, or derivative of the extracellular domain of VSP1267 (protein sequence is SEQ ID NO:2; DNA coding sequence is SEQ ID NO: 4). In other embodiments, a VSP carrier is a fragment, variant, or VSP9B10 (protein sequence is SEQ ID NO:3; DNA coding sequence is SEQ ID NO: 5). See TABLE 2.

TABLE 2

Protein and DNA Sequences of exemplary VSP carriers.

Variant-specific surface protein (VSP) 1267 (without transmembrane domain and cytosolic tail)	*Giardia lamblia* EMBL AAA29159.1

Protein Sequence (signal peptide is underlined) (SEQ ID NO: 2)

MLLIAFYLILSTFAVDCKNSGNSCEAGQCDTIGDTEICMQCNQGKVPINGICTAHSEEAVTNAGCKKNG

GTNIEESDKVCGQCGNGYFLHKGGCYKIGEAPGNLICADEASNEGARTAGVCGACKDGYYKNSDAVATA

DSCIACEDANCATCGGAGENKCTKCIDGYFVGATGNEGGCIKCDATTGPNSYKGVAGCAKCEKPKNAGP

AKCIECAADYLKTEADEQTSCVSEAVCREGKTHFPTTDSAGGNKKVCVSCGTTNNGGIENCGECTSKES

AARAGTEITCTKCSSNNLSPLGDACLTDCPAGTYAVSGDSGSVCKPCHNTCAGCQTDDRETSCTACSPG

YSLLYESEGATGRCVKECTGAFITNCADGQCTANVGGAKYCTQCKDGYAPIDGICTAVAAAGRDVSVCT

ATGGKCTACTGNYALLSGGCYNTQTLPGKSVCKAVANSNDGKCKTCANGQAPDPATNFCPLCDSTCAEC

STKNDADACTKCFPGYYKTGNKCIKCTESSENGKKIDGIPDCLSCEAPINTGPAICYVKTDGTSDDNSG

NGGDSTNKSGLST

DNA Sequence (sequence encoding the signal peptide is underlined) (SEQ ID NO: 4)

ATGTTGTTGATAGCCTTCTATCTTATATTATCTACATTTGCAGTAGATTGCAAGAATAGTGGAAATAGT

TGTGAAGCTGGCCAATGTGATACGATTGGCGATACTGAAATCTGTATGCAATGTAATCAAGGGAAAGTA

CCCATCAATGGAATATGTACAGCCCATAGTGAAGAAGCAGTCACTAACGCTGGTTGTAAGAAGAACGGG

GGTACTAATATAGAAGAAAGTGATAAGGTATGTGGACAATGTGGAAATGGCTACTTCCTGCACAAAGGC

GGATGCTATAAGATAGGAGAGGCTCCTGGCAATCTCATCTGTGCGGATGAGGCGTCAAATCCTGGTGCA

CGTACTGCAGGGGTGTGTGGTGCTTGCAAGGATGGCTATTATAAGAATTCGGATGCTGTTGCAACTGCA

GACTCCTGTATAGCATGTGAAGATGCCAACTGTGCCACATGTGGAGGAGCTGGTGAAAACAAATGTACA

AAATGTATAGACGGATACTTTGTTGGAGCAACTGGAAATGAAGGTGGGTGCATAAAATGTGACGCTACC

ACAGGGCCTAATAGCTACAAAGGAGTTGCTGGATGTGCTAAATGTGAAAAGCCAAAGAACGCTGGTCCT

GCAAAGTGCATTGAATGTGCTGCTGATTATTTGAAAACAGAAGCAGATGAACAAACGTCTTGCGTTAGC

GAAGCCGTGTGCAGAGAAGGCAAGACGCACTTCCCCACTACTGACAGCGCTGGTGGTAACAAGAAGGTA

TGCGTAAGTTGTGGCACAACGAATAATGGCGGCATAGAAAACTGTGGAGAATGCACCTCTAAGGAAAGC

GCTGCACGGGCAGGGACAGAGATCACCTGCACCAAATGCTCTAGCAATAATCTGAGCCCCCTGGGAGAC

GCGTGTCTAACAGACTGCCCTGCCGGAACGTATGCCGTTAGTGGCGACAGCGGCAGTGTCTGCAAGCCC

TGTCACAACACGTGCGCCCGCTGCCAGACCGACGACAGGGAGACTTCCTGCACGGCCTGCTcCCCTGGA

TACTCCCTTCTGTATGAGTCCAACGGAGCAACTGGGAGGTGCGTCAAGGAGTGCACTGGTGCGTTCATT

ACCAACTGTGCGGACGGGCAGTGCACGGCTAACGTCGGGGGTGCGAAGTACTGCACCCAGTGCAAGGAC

GGGTACGCCCCGATCGACGGGATCTGTACAGCGGTGGCAGCTGCCGGGAGAGACGTGAGCGTGTGCACG

GCCACAGGTGGCAAGTGCACGGCATGTACAGGCAACTATGCGTTATTATCAGGTGGATGTTATAACACA

CAAACACTTCCTGGAAAGTCAGTATGTAAAGCCGTGGCTAATAGCAATGACGGGAAATGCAAAACATGT

GCCAATGGTCAAGCACCAGATCCTGCTACTAATTTCTGCCCATTGTGTGATTCAACTTGTGCAGAATGT

TCAACTAAAAATGATGCTGATGCTTGTACAAAATGTTTTCCAGGATACTATAAAACAGGAAATAAGTGT

ATCAAATGTACAGAAAGTAGTAATAACGGAAAAAAGATCGATGGAATACCTGATTGTTTAAGTTGTGAA

GCACCGATTAATACTGGTCCTGcCATCTGCTACGTTAAAACGGATGGCACTAGCGATGATAACAGCGGC

AATGGTGGAGACAGCACCAACAAGAGCGGCCTTTCCACTGGC

TABLE 2-continued

Protein and DNA Sequences of exemplary VSP carriers.

Variant-specific surface protein (VSP) WB/9B10-B	*Giardia lamblia*) EMBL AAK97086.1

Protein Sequence (SEQ ID NO: 3)

MFMSFVLAGVLVQIAWAGKATERAAQCADNTNCAEEACNVLIGGKLYCSRCNTGFVPINGQCADKEGAT

DQCKDGSGGDTADQTCGQCAEQTFMYKGGCYEAAQQPGQTMCQAADAGVCTQAAQGYFIPPGADASHQS

VIPCGDEEGITVKNDKKYKGVLHCTRCYAPTEAADANAKAATCTACGDSKIVKTAKDSATSCVTEEECT

GTKTCKTCAEGTSDGCATCEKGADGAVACKTCGSNKKVQPNKKGCIAKCPETVSAEKDGVCECVEGYVP

DNAGTGCTKKPDPQCNTPGCKTCSEPKTSKEVCTECEDPKALTPTGQCIYGCEHLEGYYEGTSEGGKKA

CKKCEVENCLLCNGQGQCETCKDGYYKSGAACAKCNTSCKTCANGNSNGCTSCEPKQVLSYEGEGTGTC

KPGCKPVSGGKDGTCKSCDLNIDGTSYCSACNVGTEYPENGVCVKKSARTASCQAEPSNGVCGTCARGF

FRMNGGCYETTKLPGKSVCEEVASAGDTCQTPADGYKLNNGALITCSAGCKTCTSQDQCDTCKAGYAKT

GGNTKKCVPCATGCSECNADDATKCTVCAAGYYLSKEKCIACDKSDGGSITGVANCANCAPPTNNKGPV

LCYLIQNTNRSGLSTG

DNA Sequence (SEQ ID NO: 5)

ATGTTTGGCAGTTTTGTTCTCGCGGGGGTACTCGTCCAGATTGCATGGGCAGGAAAAGCAACAGAGCGC

GCGGCTCAATGCGCAGATAACACTAATTGCGCAGAGGAAGCATGCAACGTTCTGATCGGTGGTAAATTG

TATTGCTCTCGATGTAACACAGGATTTGTTCCTATCAATGGACAATGTGCAGACAAAGAAGGTGCAACA

GATCAGTGCAAAGATGGCTCCGGAGGCGATACAGCTGATCAGACCTGTGGACAGTGCGCCGAGCAGACT

TTCATGTACAAGGGCGGCTGTTACGAAGCAGCCCAGCAGCCCGGACAGACCATGTGTCAGGCGGCAGAT

GCTGGAGTATGCACACAAGCCGCGCAAGGATACTTCGTGCCGCCGGGCGCAGACGCCTCTCACCAATCG

GTCATACCATGCGGAGACGAAGAGGGAATAACAGTTAAGAACGATAAAAAGTACAAGGGCGTGCTGCAC

TGCACTCGGTGTTACGCTCCCACAGAAGCAGCAGATGCTAACGCCAAGGCCGCCACGTGTACTGCGTGC

GGCGATAGCAAGATCGTCAAGACAGCCAAGGACTCAGCCACCTCCTGCGTGACAGAAGAAGAGTGCACC

GGCACCAAGACGTGCAAGACGTGCGCCGAGGGGACCTCCGACGGGTGTGCGACGTGCGAGAAGGGCGCC

GATGGAGCAGTCGCCTGCAAGACGTGCGGGTCTAATAAGAAGGTCCAGCCAAACAAGAAGGGGTGCATA

GCAAAGTGCCCGGAGACGGTGAGTGCCGAGAAGGATGGCGTTTGTGAGTGCGTCGAGGGCTACGTTCCC

GACAACGCGGGCACCGGGTGCACGAAGAAGCCCGACCCCCAGTGCAACACCCCCGGCTGCAAGACGTGC

AGTGAGCCGAAGACAAGCAAGGAGGTGTGCACAGAGTGCGAAGACCCCAAGGCCCTCACGCCCACGGGC

CAGTGCATCTACGGTTGTGAGCACCTGGAAGGCTACTACGAGGGCACCAGCGAGGGGGGCAAGAAGGCC

TGCAAGAAGTGCGAGGTCGAGAACTGCCTCCTGTGCAACGGGCAAGGACAGTGCGAGACCTGCAAGGAC

GGGTACTACAAGAGCGGAGCCGCCTGTGCCAAGTGCAATACCTCGTGCAAGACGTGCGCGAACGGGAAC

TCCAACGGGTGCACGAGCTGCGAGCCTAAGCAGGTCCTCAGCTACGAAGGAGAGGGCACGGGGACGTGC

AAGCCAGGCTGCAAGCCAGTGAGCGGCGGCAAGGATGGAACGTGCAAGAGCTGCGACCTGAACATAGAC

GGGACAAGCTACTGTTCTGCCTGTAACGTGGGCACGGAGTATCCAGAGAACGGCGTGTGCGTCAAGAAG

TCGGCCCGCACAGCCTCCTGCCAGGCAGAACCGAGCAATGGTGTGTGCGGGACATGTGCAAGGGGCTTC

TTCCGCATGAACGGGGGCTGCTACGAAACGACCAAACTCCCTGGAAAGAGCGTCTGTGAGGAGGTAGCA

TCGGCCGGCGATACCTGTCAGACTCCGGCCGACGGATACAAGCTGAATAATGGCGCGCTCATCACTTGC

TCGGCCGGATGTAAGACGTGCACCAGCCAGGACCAGTGCGACACGTGTAAGGCTGGATATGCTAAGACT

GGCGGTAACACTAAGAAGTGCGTTCCCTGCGCCACTGGGTGCTCCGAGTGCAATGCGGACGACGCCACC

AAGTGCACGGTGTGCGCTGCAGGGTACTACCTGTCCAAAGAAAAGTGCATAGCATGCGACAAGAGCGAC

TABLE 2-continued

Protein and DNA Sequences of exemplary VSP carriers.
GGCGGATCCATCACCGGCGTCGCCAACTGCGCCAACTGCGCTCCCCCAACCAACAATAAAGGGCCTGTC
CTCTGCTACCTCATACAGAACACCAACAGGAGCGGGCTTTCCACG

VSP Carriers from VSP-like Domains

In some embodiments, a VSP carrier comprises a VSP sequence chosen from among VSP-like domains, fragments, variants or derivatives thereof from microorganisms other that *Giardia*. These VSP-like proteins share sequence homology and biochemical properties with *Giardia* VSPs. In some embodiments, VSP-like sequences selected to be used as VSP carriers comprise multiple CXXC (SEQ ID NO:589) motifs. In some embodiments, such multiple CXXC (SEQ ID NO:589) motifs are separated by 5 to 8 amino acids.

Alignment of the sequence of the extracellular domain of the *Giardia* VSP1267, used herein as an exemplary VSP carrier, with other VSP-like molecules sequences has led to observe the presence of multiple CXXC (SEQ ID NO:589) motifs, notably separated by 5 to 8 amino acids, in proteins belonging to *Paramecium, Tetrahymena* and *Entamoeba* species. Thus, representative fragments of primary sequences of surface kinases of *Entamoeba* sp., and surface proteins of *Paramecium* sp. and *Tetrahymena* sp. predict a conserved domain containing CXXC (SEQ ID NO:589) motifs in a VSP-like architecture (compared with *Giardia* VSP 1267, 9B10 (SEQ ID NO:3), and H7 as responsible for resistance to pH, temperature and proteolytic digestion).

In one embodiment, the *Tetrahymena* microorganism is *Tetrahymena thermophila*. In another embodiment, the *Entamoeba* microorganism is *Entamoeba histolytica*. In another embodiment, the *Paramecium* microorganism is *Paramecium tetraurelia*.

In one embodiment, the VSP carrier comprises the extracellular domain of a VSP-like protein, or a fragment, variant or derivative thereof (since said extracellular domain is the amino-terminal cysteine rich region comprising multiple CXXC (SEQ ID NO:589) motifs of the *Giardia* VSP protein). In another embodiment, the VSP carrier comprises only the extracellular domain of a VSP-like protein, or fragment, variant, or derivative thereof.

Thus, in some embodiments, the VSP carrier is a fragment, analog or derivative of a VSP-like protein comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 CXXC (SEQ ID NO:589) mmotifs. In some embodiments, the VSP carrier comprises at least about 40, at least about 50, at least about 60, at least about 70, at least 80, about 90 or at least about 100 CXXC (SEQ ID NO:589) motifs from a VSP-like protein.

VSPs as Therapeutic Agent Carriers

The VSP carriers can be used to deliver therapeutic agents to a subject in need thereof. Thus, in some embodiments, the present disclosure provides a therapeutic composition comprising a VSP carrier and a therapeutic agent. This therapeutic composition can be formulated, for example, for oral administration. In some embodiments, the therapeutic composition is formulated for mucosal administration. As disclosed above, the VSP carrier can comprise without limitation a VSP, a VSP-like protein, a VSP or VSP-like protein fragment, a VSP or VSP-like protein derivative, or a combination of two or more of said VSP carriers. In specific embodiments, the VSP carrier comprises a VSP from *Giardia* (e.g., VSP1267) or a fragment thereof, e.g., a VSP extracellular domain or a fragment of such extracellular domain.

In some embodiments, the VSP carrier comprises a VSP protein sequence and further comprises a heterologous moiety, for example, a purification tag such as a Hi6 tag. In other embodiments, the heterologous moiety can be a protein, peptide, polymer, etc. that can improve a pharmokinetic or pharmacodynamic property such as half-life. In a specific embodiment, the VSP carrier is SEQ ID NO:1, i.e., the extracellular domain of *Giardia* VSP1267, including the N-terminal signal peptide, and a C-terminal His6 tag.

Therapeutic agents that can be delivered by a VSP carrier include biological agents. The term "biological agent" includes both proteins and non-protein therapeutic agents. Exemplary non-protein therapeutic agents include polysaccharides, lipids, drugs (e.g., small molecule drugs), nucleic acids (e.g., oligonucleotides), lipopolysaccharides, ribozymes, genetic materials, prions, viruses, etc.

In some embodiments of the present invention, the therapeutic agent is a pharmacologically active polypeptide. In some embodiments, the polypeptide is a bioactive peptide, e.g., a cytokine, an interleukin (e.g., IL-2 or IL-10), a hormone (e.g., parathormone), a growth factor, or a receptor. In specific embodiments, the bioactive peptides can be, without limitation, insulin, human growth hormone, glucagon, parathormone, IL-2, IL-10, as well as fragments, analogs, derivatives or variants thereof, or combinations of two or more of these bioactive peptides. The examples provided above are non-limiting, and it is contemplated that a VSP carrier can be used to delivered other bioactive peptides and proteins. For example, VSP carriers can be used for oral or mucosal delivery of proteins comprising antigen-binding domains such antibodies and fragments thereof (e.g., scFv's or scFv-comprising molecules).

In some specific embodiments, the bioactive peptide is insulin, e.g., a natural insulin, a recombinant insulin, or an insulin analog. In some embodiments, the insulin analog is a fast-acting insulin (e.g., insulin aspart), a long-lasting insulin (e.g., insulin glargine) or a combination thereof. Numerous insulin analogs are known in the art.

Therapeutics agents can also include classical low molecular weight therapeutic agents commonly referred to as drugs, including but not limited to antineoplastic, immunosupressants, antioproliferatives, antithrombins, antiplatelet, antilipid, anti-inflammatory, angiogenic, antiangiogenic, vitamins, ACE inhibitors, vasoactive substances, antimitotics, metalloproteinase inhibitors, NO donors, estradiols, or antisclerosing agents, alone or in combination. In some embodiments, the drug is a drug poorly soluble under aqueous conditions, for example an antibiotic.

The therapeutic agent can also be a compound that needs to be activated in order to be therapeutically active, e.g., a prodrug or a zymogen. In such embodiments, the therapeutic agent is metabolized into the desired drug or biological agent after it has been administered to a subject in combination with a VSP carrier.

In some embodiments, the VSP carrier is bound directly to the therapeutic agent. In other aspects, the VSP carrier is bound to a vector particle containing the therapeutic agent. Accordingly, the vector particle can be a viral particle, a virus-like particle (VLP), a nanoparticle, or a liposome. In one particular aspect, the vector particle is a viral particle displaying at its surface the therapeutic agent. In another aspect, the vector particle is a viral which does not display the therapeutic agent on its surface. In one particular aspect, the vector particle is a VLP displaying at its surface the therapeutic agent. In another aspect, the vector particle is a VLP encapsulating the therapeutic agent. In one particular aspect, the vector particle is a nanoparticle displaying at its surface the therapeutic agent. In another aspect, the vector particle is a nanoparticle encapsulating the therapeutic agent. In one particular aspect, the vector particle is a liposome displaying at its surface the therapeutic agent. In another aspect, the vector particle is a liposome encapsulating the therapeutic agent. In another particular aspect, the therapeutic agent is contained within the surface of the vector particle, e.g., within a lipid bilayer in a liposome.

In a particular embodiment, the vector particle is a virus-like particle (VLP). Where virus-like particles are being used, they can be prepared according to techniques known in the art and for example as described in Intl. Pat. Appl. Publ. No. WO 2002/34893, which is incorporated therein by reference in its entirety. In some embodiments, the VLP displays at its surface a VSP carrier and a therapeutic agent. Thus, in some embodiments, a VSP carrier can be bound to a therapeutic agent exposed at the surface of the VLP.

The VSP carrier according to the invention can form a protecting surface (as it occurs naturally in the parasite trophozoites) that allows for the correct delivery of the therapeutic agent into the mucosa (e.g., intestinal mucosa), without suffering degradation in the digestive tract.

In certain embodiments, the VSL carriers provided herein are not covalently attached to the therapeutic agents via peptidic bonds. Thus, prior to administration, a VSP carrier is "combined" with at least one therapeutic agent. As disclosed above, the term "combine" refers to the process of admixing two or more components (e.g., a VSP carrier and a therapeutic agent) such that contact between the components occur and such contact allows the binding of the two or more components.

In some embodiments, a VSP carrier can be combined with one therapeutic agent. In other embodiments, a VSP carrier can be combined with more than one therapeutic agent. In some embodiments when a VSP carrier is combined with more than one therapeutic agent, the VSP carrier can bind to only one of the therapeutic agents. In other embodiments, the VSP carrier can bind to more than one of the therapeutic agents.

In some embodiments, two or more VSP carriers can be combined with one therapeutic agent. In other embodiments, two or more than two VSP carriers can be combined with more than one therapeutic agent. In some embodiments, when two or more than two VSP carriers are combined with more than one therapeutic agent, each VSP carrier can bind to only one of the therapeutic agents. In other embodiments, each VSP carrier can bind to more than one therapeutic agent.

In some embodiments, the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP carrier:therapeutic agent) ranges from about 10:1 to about 1:10. In other embodiments, the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP carrier:therapeutic agent) ranges from about 3:1 to about 1:3. In some embodiments, the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP carrier:therapeutic agent) is 3:1. In other embodiments, the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP carrier:therapeutic agent) is 1:1.

In some embodiments, the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP carrier:therapeutic agent) is about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1. In some embodiments, the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP:therapeutic agent) is higher that 10:1. In some embodiments, the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP carrier:therapeutic agent) is about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, or about 1:10. In some embodiments the molecule to molecule ratio of VSP carrier to therapeutic agent (VSP:therapeutic agent) is lower than 1:10.

In some aspects, the VSP carrier and the therapeutic agent are co-administered, i.e., they are administered simultaneously to the subject, so they combine at the time of administration. In some aspects, the VSP carrier and the therapeutic agent are combined prior to administration. In some aspects, the combination of VSP carrier and therapeutic agent can take place at least about 1 minute, at least about 2 minutes, at least about 3 minutes, at least 4 minutes, at least about 5 minutes, at least about 10 minutes, at least about 15 minutes, at least about 20 minutes, at least about 25 minutes, at least about 30 minutes, at least about 45 minutes, at least about 1 hours, at least about 2 hours, at least 3 hours, at least about 4, or at least 6 hours prior to administration. In some embodiments, the VSP carrier and the therapeutic agent are combined at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, or at least 7 days prior to administration. In some embodiments, a VSP carrier and a therapeutic agent are combined in a stable form that can be used days, weeks or months after combining the VSP carrier and the therapeutic agent.

VSP as an Insulin Carrier

In certain aspects, a VSP carrier can be combined with an insulin for oral or mucosal administration to a subject.

As used herein, the term "insulin" comprises insulin analogs, natural extracted mammalian insulin (e.g., human insulin), recombinantly produced mammalian insulin (e.g., human insulin), insulin extracted from bovine and/or porcine sources, recombinantly produced porcine and bovine insulin, insulin produced in transgenic animals, and mixtures of any of these insulin products. The term is intended to encompass the polypeptide normally used in the treatment of diabetics in a substantially purified form, but encompasses the use of the term in its commercially available pharmaceutical form, which includes additional excipients. The insulin used to combine with a VSP carrier can be recombinantly produced and can be dehydrated (completely dried) or in solution.

The term "insulin analog" refers to any form of "insulin" as defined above, where one or more of the amino acids within the polypeptide chain has been replaced with an alternative amino acid and/or where or more of the amino acids has been deleted or wherein one or more additional amino acids has been added to the polypeptide chain or amino acid sequences, which act as insulin in decreasing blood glucose levels. In general, the term "insulin analogs" includes, e.g., "insulin lispro" analogs as disclosed, e.g., in U.S. Pat. No. 5,547,929, which is herein incorporated by reference in its entirety; insulin analogs including LysPro insulin and Humalog insulin and other "super insulin" analogs, wherein the ability of the insulin analog to affect

serum glucose levels is substantially enhanced as compared with conventional insulin as well as hepato selective insulin analogs which are more active in liver than in adipose tissue. The term "insulin analogs" also includes chemically and enzymatically modified insulins (e.g., mammalian insulins chemically converted into human insulin), NPH insulin (e.g., the intermediate-acting isophane insulin), insulin aspart, insulin glulisine, insulin glargine, insulin detemir, insulin degludec, etc.

In some embodiments, insulin analogs are monomeric insulin analogs, which are insulin-like compounds used for the same general purpose as insulin, such as insulin lispro, e.g., any compounds which are administered to reduce blood glucose levels.

"Insulin analogs" are well known compounds. Insulin analogs are known to be divided into two categories: animal insulin analogs and modified insulin analogs (pages 716-20, chapter 41, Nolte M. S, and Karam, J. H., "Pancreatic Hormones & Antidiabetic Drugs" In Basic & Clinical Pharmacology, Katzung, B. G., Ed., Lange Medical Books, New York, 2001). Historically, animal insulin analogs include porcine insulin (having one amino acid different from human insulin) and bovine insulin (having three amino acids different from human insulin) which have been widely used for treatment of diabetes. Since the development of genetic engineering technology, modifications are made to create modified insulin analogs, including fast-acting insulin analogs or longer acting insulin analogs. Several insulin analog molecules have been on the market prior to the filing date of the subject application. For example, Eli Lilly markets a fast-acting insulin analog called "lispro" under the trade name HUMALOG® and Novo Nordisk sells another fast-acting insulin analog called "aspart" under the trade name NOVOLOG®. In addition, Aventis markets a long-acting insulin analog called "glargine" under the trade name LANTUS® and Novo Nordisk markets another long-acting insulin analog called "detemir" under the trade name LEVEMIR®. Table 41-4 of the Nolte and Karam (2001) reference cited above provides a non-limiting list of the wide range of types of molecules generically referred to as insulin.

The term insulin also encompasses insulin as defined above covalently coupled to one or more heterologous moieties that can improve pharmacokinetic and/or pharmacodynamic properties over native insulins, e.g., PEGylated insulins (see, e.g., U.S. Pat. No. 6,890,518). See also, U.S. Pat. Nos. 7,049,286; 7,470,663; 6,890,518; and U.S. Appl. Pub. Nos. US2008/0139784; US2011/0281791; US2009/0036353; US20110020871; US2009/0239785.

VSP as a Glucagon Carrier

In certain aspects, a VSP carrier can be combined with glucagon for oral or mucosal administration to a subject.

As used herein, the term "glucagon" comprises glucagon analogs, natural extracted mammalian glucagon (e.g., human glucagon), recombinantly produced mammalian glucagon (e.g., human glucagon), glucagon extracted from bovine and/or porcine sources, recombinantly produced glucagon, glucagon produced in transgenic animals, and mixtures of any of these glucagon products. The term is intended to encompass the polypeptide normally used in the treatment of hypoglycemia (?) in a substantially purified form, but encompasses the use of the term in its commercially available pharmaceutical form, which includes additional excipients. The glucagon used to combine with a VSP carrier can be recombinantly produced. In some embodiments, glucagon can be dehydrated (completely dried) or in solution.

VSP as a Growth Hormone Carrier

In certain aspects, a VSP carrier can be combined with a growth hormone, e.g., human growth hormone, for oral or mucosal administration to a subject.

The terms "growth hormone (GH)" refers generally to growth hormones secreted by the pituitary gland in mammals. Although not an exhaustive list, examples of mammals include human, apes, monkey, rat, pig, dog, rabbit, cat, cow, horse, mouse, rat and goat. In some embodiments of the present invention, the mammal is a human.

The terms "human growth hormone" and "hGH" are used interchangeably and refer to a protein having an amino acid sequence, structure and function characteristic of native human growth hormone. As used herein, hGH also includes any isoform of native human growth hormone, including but not limited to, isoforms with molecular masses of 5, 17, 20, 22, 24, 36 and 45 kDa (see, e.g., Haro et al., J. Chromatography B, 720, 39-47 (1998)). Thus, the term hGH includes the 191 amino acid sequence of native hGH, somatotropin, and the 192 amino acid sequence containing an N-terminal methionine (Met-hGH) and somatrem (see, e.g., U.S. Pat. Nos. 4,342,832 and 5,633,352). hGH can be obtained by isolation and purification from a biological source or by recombinant DNA methods. Met-hGH is typically prepared by recombinant DNA methodology.

The term "human growth hormone" also encompasses human growth hormone derivatives. The term "human growth hormone derivative" refers to a protein that differs by at least about 1% but not by more than about 20% from the amino acid sequence of the 191 amino acid sequence of hGH or the 192 amino acid-sequence of Met-hGH. For example, the derivative can differ by about 1% to about 20%, about 2% to about 15%, or about 5% to about 10% from the 191 amino acid sequence of hGH or the 192 amino acid-sequence of Met-hGH; the protein can differ by about 1%, about 2%, about 3%, about 4%, about 51%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% from the 191 amino acid sequence of hGH or the 192 amino acid-sequence of Met-hGH. The differences between the derivative and the 191 amino acid hGH or the 192 amino acid Met-hGH amino acid sequence can be one or more substitutions (e.g., conservative or non-conservative substitutions), deletions, additions (e.g., insertions or amino- or carboxy-terminal additions)), modifications, or combinations thereof.

In some embodiments, an hGH derivative maintains a biological activity and/or a chemical and/or physical property of the 191 amino acid hGH or the 192 amino acid Met-hGH amino acid sequence. Likewise, in some embodiments, a formulation containing a derivative (e.g., a formulation of poly-Arg complexed crystalline hGH derivative) possesses a chemical and/or physical property of a similarly-prepared formulation containing the 191 amino acid hGH or the 192 amino acid Met-hGH amino acid sequence (e.g., a formulation of poly-Arg complexed crystalline hGH).

In various embodiments of the present disclosure, human growth hormone derivatives comprise organic cations of hGH or Met-hGH, substitution, deletion and insertion variants of biologically synthesized hGH or Met-hGH proteins, post-translationally modified hGH and Met-hGH proteins, including—without limitation—deamidation, phosphorylation, glycoslylation, acetylation, aggregation and enzymatic cleavage reactions (see, e.g., Haro et al., J. Chromatography B, 720, 39-47 (1998)), chemically modified hGH or Met-hGH proteins derived from biological sources, polypeptide analogs and chemically synthesized peptides con-

taining amino acid sequences analogous to those of hGH or Met-hGH. Methods used to prepare hGH or Met-hGH include isolation from a biological source, recombinant DNA methodology, synthetic chemical routes or combinations thereof. Genes that encode for different DNA sequences of hGH include hGH-N and hGH-V (see, e.g., Haro et al., J. Chromatography B, 720, 39-47 (1998); Bennani-Baiti et al., Genomics, 29, 647-652 (1995)). hGH is commercially available in lyophilized form and is typically produced by recombinant DNA methods.

Production of VSP Carriers

Recombinant expression of the VSP carriers can be achieved through the construction of an expression vector containing a polynucleotide that encodes a VSP carrier. Once a polynucleotide encoding a VSP carrier has been obtained, the vector for the production of the VSP carrier can be produced by recombinant DNA technology using techniques well known in the art.

Methods for preparing a protein by expressing a polynucleotide containing a VSP carrier-encoding nucleotide sequence are known in the art. Methods that are well known to those skilled in the art can be used to construct expression vectors containing VSP carrier coding sequences and appropriate transcriptional translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The disclosure, thus, provides replicable vectors comprising a nucleotide sequence encoding a VSP carrier operably linked to a promoter.

An expression vector can be transferred to a host cell by conventional techniques and the transfected cells can then be cultured by conventional techniques to produce a VSP carrier. Thus, the invention includes host cells containing a polynucleotide encoding a VSP carrier, operably associated with a promoter. Suitable host cells include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*), fungal cells, mammalian cells, or insect cells.

A variety of host-expression vector systems can be utilized to express the VSP carrier of the present disclosure. Such host-expression systems represent vehicles by which the coding sequences of interest can be produced and subsequently purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, express a VSP carrier in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing VSP carrier coding sequences, fungal cell system (e.g., *Saccharomyces* or *Pichia*), mammalian cell systems (e.g., COS, CHO, BHK, 293, NSO, and 3T3 cells), or insect cell systems. Once a VSP carrier has been produced by recombinant expression, it can be purified by any method known in the art for purification of a protein.

In some embodiments, a DNA encoding a VSP carrier is codon optimized for expression in an insect protein expression system, e.g., a baculovirus expression system. In some embodiments, a VSP carrier is expressed in an insect protein expression system, e.g., a baculovirus expression system.

Pharmaceutical Compositions

In another aspect, the present disclosure provides a therapeutic composition including, but not limited to, a pharmaceutical composition, containing one or more than one VSP carrier combined with one or more than one therapeutic agents, formulated together with a pharmaceutically acceptable excipient. Such compositions can include one or a combination of two or more different VSP carriers. For example, a pharmaceutical composition can comprise a combination of VSP carriers that bind to the same therapeutic agent or to more than one therapeutic agent. These therapeutic agents can have complementary activities. In a specific aspect, a pharmaceutical composition comprises a single VSP carrier. In a specific embodiment, a pharmaceutical composition comprises more than one VSP carriers.

Pharmaceutical compositions comprising one or more VSP carriers also can be administered in combination therapy. For example, the combination therapy can include a pharmaceutical composition which comprises at least one VSP carrier combined with at least one therapeutic agent, combined with at least one other therapy wherein the therapy can be immunotherapy, chemotherapy, radiation treatment, or drug therapy. Pharmaceutical compositions of the invention can include one or more pharmaceutically acceptable salts.

Examples of suitable aqueous and non-aqueous carriers that can be employed in contemplated pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

In another aspect, pharmaceutical compositions comprising a VSP carrier can also contain agents such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms can be ensured both by sterilization procedures and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It can also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption can be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the pharmaceutical compositions of the invention.

Actual dosage levels of the active ingredients in pharmaceutical compositions comprising a VSP carrier can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A therapeutically effective dosage of pharmaceutical composition comprising a VSP carrier can be indicated by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. A therapeutically effective dose can also prevent or delay onset of disease. Accordingly, any clinical or biochemical monitoring assay can be used to determine whether a particular treatment is a therapeutically effective dose. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

Therapeutic compositions comprising a VSP carrier are particularly well suited for oral administration. Alternatively, therapeutic compositions comprising a VSP can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, buccally, vaginally, rectally, sublingually or topically. Of course, therapeutic compositions comprising a VSP carrier can be administered via one or more alternative routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

In certain embodiments, therapeutic compositions comprising VSP carriers are formulated for an oral or a mucosal administration. The doses used for the oral or a mucosal administration can be adapted as a function of various parameters, and in particular as a function of the mode of the relevant pathology, or alternatively of the desired duration of treatment.

Upon formulation, pharmaceutical compositions comprising VSP carriers can be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of, e.g. tablets or other solids for oral or a mucosal administration; time release capsules; and any other form currently used. Accordingly, the pharmaceutical composition may be in the form of a spray, an aerosol, a mixture, a suspension, a dispersion, an emulsion, a gel, a paste, a syrup, a cream, an ointment, implants (ear, eye, skin, nose, rectal, and vaginal), intramammary preparations, vagitories, suppositories, or uteritories). In certain embodiments, the use of liposomes is contemplated. The formation and use of liposomes are known to those of skill in the art.

More particularly, the pharmaceutical composition is formulated so that the therapeutic agent in the therapeutic composition of the invention is resistant to enzymatic and chemical degradation of the upper gastrointestinal tract, when necessary. Moreover, in certain embodiments, a VSP carrier should be able to attach to cells, more particularly epithelial cells of the gut.

Methods

The VSP carriers of the present disclosure have in vitro and in vivo therapeutic and diagnostic utility. For example, the VSP carriers can be used to administer therapeutic agents or diagnostic reagents to cells in culture, e.g., in vitro or ex vivo, or in a subject, e.g., in vivo, to treat, prevent or diagnose a variety of disorders. A disease, a disorder or physiologic conditions considered in the invention can be, but it is not limited to, hormone deficiencies, cancers, immunological diseases, autoimmune diseases, allograft rejections, viral diseases, such as influenza or AIDS, parasitic diseases, bacterial infections, or allergies.

The present disclosure provides a method of delivering a therapeutic agent to a target location in a subject comprising the administration of a therapeutic composition comprising a VSP carrier and a therapeutic agent. In some specific embodiments of the disclosed method of delivering, the VSP carrier is the VSP1267 carrier and the therapeutic agent is a bioactive peptide such as insulin, glucagon, or hGH.

Also provided is a method of treating a disease or condition in a subject comprising administering to said subject an effective amount of a therapeutic composition comprising a VSP carrier and a therapeutic agent. In some embodiments, the disease or condition is a hormone deficiency. In specific embodiments, the hormone deficiency is an insulin deficiency. In some embodiments, the insulin deficiency is type 1 diabetes. In some specific embodiments, the method of treating the hormone deficiency comprises administering the VSP1267 carrier and a bioactive peptide such as insulin, glucagon, or hGH.

The present disclosure also provides a method of treating a disease or condition in a subject comprising combining a VSP carrier and a therapeutic agent, wherein the VSP carrier can bind to the therapeutic agent, and administering an effective amount of the combination of VSP carrier and therapeutic agent to the subject. In some specific embodiments, the VSP carrier is the VSP1267 carrier and it is combined with a therapeutic agent which is a bioactive peptide such as insulin, glucagon, or hGH.

The present disclosure also provides a method of making an orally or mucosally deliverable composition, comprising combining a VSP carrier and a therapeutic agent, wherein the VSP carrier can bind to the therapeutic agent. In some specific embodiments, the orally or mucosally deliverable composition comprises the VSP1267 carrier combined with a therapeutic agent which is a bioactive peptide such as insulin, glucagon, or hGH.

Also disclosed herein is a method of making an injectable composition suitable for oral or mucosal administration comprising combining a VSP carrier and a therapeutic agent, wherein the VSP carrier can bind to the therapeutic agent. In some specific embodiments, the orally or mucosally deliverable injectable composition comprises the VSP1267 carrier combined with a therapeutic agent which is a bioactive peptide such as insulin, glucagon, or hGH.

Also provided in the present disclosure is a method of increasing resistance of a therapeutic agent to enzymatic degradation comprising combining a VSP carrier and a therapeutic agent, wherein the VSP carrier can bind to the therapeutic agent, and wherein combining the VSP carrier and the therapeutic agent results in improved resistance of the therapeutic agent to enzymatic degradation. In some specific embodiments, the VSP carrier is the VSP1267 and the therapeutic agent is a bioactive peptide such as insulin, glucagon, or hGH.

The present disclosure also provides a method of increasing the resistance of a therapeutic agent to pH denaturation comprising combining the therapeutic agent with a VSP

carrier, wherein the VSP carrier can bind to the therapeutic agent, and wherein combining the VSP carrier and the therapeutic agent results in improved resistance of the therapeutic agent to pH denaturation. In some specific embodiments, the VSP carrier is the VSP1267 and the therapeutic agent is a bioactive peptide such as insulin, glucagon, or hGH.

The present disclosure also provides a method of increasing simultaneously the resistance of a therapeutic agent to enzymatic degradation and its resistance to pH denaturation comprising combining the therapeutic agent with a VSP carrier, wherein the VSP carrier can bind to the therapeutic agent, and wherein combining the VSP carrier and the therapeutic agent results in an increased resistance of a therapeutic agent to enzymatic degradation and increased resistance to pH denaturation. In some embodiments, the combination of the VSP carrier and the therapeutic agent increases the therapeutic agent's resistance to pH-mediated degradation when exposed to a pH ranging between about 1 and about 2, or between about 2 and about 3, or between about 3 and about 4, or between 4 and about 5, or between about 5 and about 6, or between about 6 and 7, or between about 7 and about 8, or between about 8 and about 9, or between about 9 and about 10, or between about 10 and about 11, over between about 11 and about 12, or between about 12 and about 13, or between about 13 and about 14. In some specific embodiments, the VSP carrier is the VSP1267 and the therapeutic agent is a bioactive peptide such as insulin, glucagon, or hGH.

Also provided is a method of improving the attachability of a therapeutic agent to mucosal epithelial cells comprising combining a therapeutic agent with a VSP carrier, wherein the VSP carrier can bind to the therapeutic agent, and wherein combining the VSP carrier and the therapeutic agent results in improved attachability of the therapeutic to mucosal epithelial cells. In some specific embodiments, the VSP carrier is the VSP1267 and the therapeutic agent is a bioactive peptide such as insulin, glucagon, or hGH. In some embodiments, the mucosal epithelial cells are intestinal epithelial cells. In other embodiments, the mucosal epithelial cells as gastric epithelial cells. In other embodiments, the mucosal epithelial cells are oral epithelial cells. Mucosal delivery, i.e., delivery of a therapeutic agent to mucous tissue by a VSP carrier refers, e.g., to delivery to bronchial and other respiratory tract mucosal tissues, gingival, lingual, nasal, oral, gastrointestinal, and genitourinary tract mucosal tissues.

The invention also provides methods of using VSP carriers in diagnostics. In some embodiments, a VSP carrier can be combined with one or more than one diagnostic reagents. The invention also provides methods of imaging specific targets using VSP carriers. In one embodiment, a VSP carrier is combined imaging agents such as green-fluorescent proteins, other fluorescent tags (Cy3, Cy5, Rhodamine and others), biotin, or radionuclides to be used in methods to image the presence, location, or progression of a specific target. In some aspects, the method of imaging a target comprising a VSP carrier is performed by MRI, PET scanning, X-ray, fluorescence detection or by other detection methods known in the art.

Therapies comprising the use of VSP carriers can be combined with conventional therapies suitable for the prevention, treatment, reduction or amelioration of disease or symptoms thereof. Exemplary conventional therapies can be found in the Physician's Desk Reference (56th ed., 2002 and 57th ed., 2003). In some embodiments, therapies using VSP carriers can be combined with chemotherapy, radiation therapy, surgery, immunotherapy with a biologic (e.g., an antibody or antigen-binding fragment thereof, or a peptide, e.g., a bioactive peptide), small molecules, or another therapy known in the art. In some embodiments, the combinatorial therapy is administered together with the therapy comprising the use of VSP carriers. In other embodiments, the combinatorial therapy is administered separately from the therapy comprising the use of VSP carriers.

The present disclosure also provides methods of monitoring disease progression, relapse, treatment, or amelioration using the VSP carriers. In one embodiment, methods of monitoring disease progression, relapse, treatment, or amelioration is accomplished by the methods of imaging, diagnosing, or contacting a compound/target with a VSP carrier as presented herein.

The present disclosure also provides a method to increase the solubility of a poorly soluble drug (e.g., a small molecule drug) by combining it with a VSP carrier, wherein the binding of the poorly soluble drug to the VSP carrier increases the solubility of the drug. In some embodiments, the poorly soluble drug is a small molecule drug used to treat a hormonal imbalance (e.g., an antidiabetic small molecule drug). In other embodiments, the poorly soluble drug is an antibiotic. In some embodiments, the antibiotic is an aminoglycoside antibiotic, e.g., amikacin. In other embodiments, the antibiotic is a glycopeptide antibiotic, e.g., vancomycin.

Kits

Also provided is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the pharmaceutical compositions disclosed herein. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. The present disclosure provides kits that can be used in the above methods of treatment and administration. In one aspect, a kit comprises a VSP carrier, preferably in a purified form, in one or more containers. In some embodiments, the kit comprises a VSP carrier combined with a therapeutic in one container. In other embodiments, the kit comprises a VSP carrier and a therapeutic agent in different containers.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All publications, patents, and patent applications referred to herein are expressly incorporated by reference in their entireties.

EXAMPLES

Example 1

VSP Resistance to Variable pH and to Digestion by Intestinal Proteases

VSPs are integral membrane proteins of protozoan parasites, e.g., *Giardia lamblia*, with a variable extracellular region rich in CXXC (SEQ ID NO:589) motifs, a unique transmembrane hydrophobic regions and a short, 5 amino acid-long cytoplasmic tail (FIG. **1**C). Each trophozoite (the active, motile feeding stage of the *Giardia* parasite) expresses a single VSP on its surface, as shown in FIGS. **1**A and **1**B. FIG. **1**A shows phase contrast (left panel) and immunofluorescence (right panel) assays showing that, from a group of *Giardia* trophozoites only one expresses on its surface a given VSP, as demonstrated by surface labeling with an anti-VSP specific monoclonal antibody, while the other cells express a different surface protein. FIG. **1**B shows an anti-VSP specific immunogold labeling of the surface of a trophozoite. It can be observed that the entire surface of the parasite is labeled, including the ventral disk and the flagella, generating a thick surface coat.

Purified VSPs are not toxic for cells when added to cultures and they are not toxic for the animals when administered by the oral route. Animals do not lose weight and have no diarrhea, which are commonly associated to *Giardia* infections (see Rivero et al., Nat. Med. 16(5):551-7 (2010)). Despite their lack of toxicity, to be useful as therapeutic agent carriers, VSPs must be able to survive the harsh conditions of the GIT.

To determine whether VSPs are resistant to variable pH and to proteolytic digestion, two different *Giardia* isolates (clonal trophozoite populations) were treated with trypsin and with variable pH and the effect of those conditions on the VSP expressed by each isolate were monitored using immunofluorescence assays.

For the trypsin resistance assay, *Giardia* parasites were resuspended in PBS at pH 7.4 and treated for 90 minutes with variable concentrations of trypsin similar of those found in the upper small intestine. Afterwards, the parasites were washed, and incubated with two monoclonal antibodies, each one recognizing the particular VSP expressed by each of the two isolates tested: the GS/M isolate and the WB isolate. The monoclonal antibody G10/4 recognized a conformational epitope in VSPH7 of the GS/M isolate. The monoclonal antibody 9B10 detected a non-conformational epitope in VSP9B10 of the WB isolate. The antibodies agglutinated the trophozoites and labeled the surface of the parasites.

It was observed that after incubation with 100 μg/ml or 200 μg/ml of trypsin, the presence of both VSP proteins was still detectable by the monoclonal antibodies, indicating that both VSPs survived trypsinization (FIG. **2**A).

For the pH resistance assay, *Giardia* parasites were resuspended in RPMI cell culture medium at variable pHs, from pH 1 to pH 10, in 1 pH unit increments (only pH 1, 3, 5 and 10 are shown in FIG. **2**B). *Giardia* parasites were kept at the different pHs for 90 minutes, washed, and then incubated with the corresponding monoclonal antibody. In all cases the VSP epitopes in VSPH7 and VSP9B10 remained intact as determined by immunofluorescence microscopy, indicating that both VSPs survived exposure to variable pH without undergoing chemical degradation (FIG. **2**B).

Resistance to proteolytic digestion was also demonstrated using Western Blot analysis of VSPs after treating trophozoites with trypsin (FIG. **3**). *Giardia* parasite clones WB-9B10, WB-1267 and GS-H7 (expressing, respectively, the VSP9B10, VSP1267 and VSPH7 VSPs) were resuspended in PBS at pH 7.4 and treated for 90 min at 37° C. with variable concentrations of trypsin (200 μg/ml and 2 mg/ml). Then, the parasites were lysed and equivalent protein amounts were applied to each lane in the SDS-PAGE gels. FIG. **3** shows that the three VSPs tested were able to survive exposure to trypsin.

These results demonstrated that VSPs are resistant to chemical and proteolytic degradation and can survive environmental conditions similar to those found in the GIT.

Example 2

VSP Attachment to Enteric Mucosa after Oral Administration

To determine whether VSPs were capable of attaching to the enteric mucosa after oral administration, in vivo assays were conducted in gerbils. A group of gerbils was infected with *Giardia* parasite clone WB-9B10 trophozoites (FIG. **4**, panel A), a second group was not infected (FIG. **4**, panel B), and a third group of gerbils was immunized with the entire repertoire of VSPs purified from transgenic trophozoites (FIG. **4**, panel C). Tissue sections from each group were incubated with the anti-VSP9B10 monoclonal antibody, detected with anti-mouse immunoglobulins labeled with horseradish peroxidase, developed with 3,3' diaminobenzidine, and counterstained with hematoxylin/eosin.

A strong difference on the level of staining of the surface of the gut epithelial cells between infected or immunized (panels A and C) compared to non-infected animal (panel B) was observed, indicating that VSPs remained attached to the enteric mucosa after oral administration.

These results, together with the results of Example 1 indicated that VSPs survived pH and enzymatic conditions in the GIT and successfully attached to gut epithelial cells. These physicochemical properties would allow VSPs to shuttle drugs through the GIT, and the prolonged stay in the GIT should allow the passage of drugs carried by the VSPs from the GIT to the bloodstream.

Example 3

Recombinant Production of VSP1267 VSP Carrier

To obtain the VSP carrier to be used for the oral administration experiments, a modified VSP protein was designed and recombinantly produced. The full-length VSP contained a cysteine-rich extracellular region containing numerous CXXC (SEQ ID NO:589) motifs. A DNA construct in which the transmembrane region and the cytoplasmic 5 residues of the VSP1267 were eliminated and a His6 protein purification tag was added at the carboxy terminal (FIG. **5**A) was generated (SEQ ID NO:1). The signal sequence is underlined in FIG. **5**A. The amino acids in the His6 protein purification tag are show in a box in FIG. **5**A.

Initially, the VSP carrier was recombinantly expressed in *E. coli* BL21 using the pET28 expression vector (Novagen). Protein production was subsequently improved by codon optimization of the recombinant DNA sequence for expression in baculovirus. The protein was expressed and purified by one step affinity purification using the His6 tag present in the carboxy terminal portion of the protein (FIG. **5**B).

Example 4

Sensitivity of Commercial Insulins to Trypsin

To test in vitro the capacity of VSP carriers to protect bioactive peptides from degradation, the capacity of recombinant VSP1267 to protect insulin from conditions similar to those present in the GIT was evaluated. Natural Insulin is a bioactive peptide with a molecular mass of 5.8-6 kDa. Prior to evaluating the capacity of the VSP to protect insulin from in vivo degradation in the GIT, the sensitivity of two commercial insulins to proteolysis by trypsin and pancreatine was tested in vitro.

Two types of insulin were tested:

LANTUS®: Insulin Glargine (Sanofi-Aventis). Differs from natural human insulin in that the amino acid asparagine at position A21 is replaced by glycine and two arginines are added to the C-terminus of the B-chain, MW: 6,063 kDa). It is long-acting insulin.

NOVORAPID®: Insulin Aspart (Novo/Nordisk). Differs from human insulin in that the amino acid, B28, which is normally proline, is substituted with an aspartic acid residue). It is fast acting insulin.

The proteolytic profiles of NOVORAPID® and LANTUS® after preincubation of the insulins with trypsin at 100, 150, 200, 500 and 1000 μg/mL, and pancreatine at enzyme: substrate ratios of 1:1 and 1:2 are shown in FIG. **6**. The insulins and their degradation products were visualized using silver staining. LANTUS® insulin was not easily degraded in the assayed experimental conditions. However, for NOVORAPID®, a trypsin dose-dependent increase in proteolytic degradation was observed.

Example 5

In Vivo Capacity of VSPs to Protect Insulin from Degradation

The combination of insulin with the VSP carrier VSP1267 was assayed to determine whether the VSP can (i) protect insulin from degradation when administered orally and (ii) promote its systemic biological action, namely the regulation of blood glucose levels. Accordingly, a sub-optimal oral dose of insulin was first determined, and then it was determined whether the combination of insulin at this sub-optimal dose with the VSP carrier could promote insulin's biological action (see FIG. **7**).

The biological activity of insulin was measured by testing its hypoglycemic capacity. Accordingly, blood glucose levels were quantified in female Balb/c 7 week-old mice left without food intake for 2 hours. After the starvation period, the mice received 1 IU, 5 IU and 50 IU oral doses of LANTUS® (FIG. **7**A) or NOVORAPID® (FIG. **7**B). Blood glucose levels were determined at the indicated time points. Blood glucose levels were also quantified after the administration of an i.v. bolus of glucose, and after subcutaneous administration of 1-5 IU of insulin as a positive control. These experiments indicated that 1 IU of insulin was a sub-optimal oral dose that could be used for the follow up experiments testing the administration of insulin in combination with a VSP carrier (see FIG. **8**).

The results shown in FIG. **8** demonstrate that the administration of insulin in combination with a VSP carrier promotes insulin action when administered by the oral route. In this experiment, female Balb/c mice, 7 week-old were left without food intake for 2 hours and then received doses of insulin, LANTUS® (FIG. **8**A) and NOVORAPID® (FIG. **8**B), at the suboptimal dose identified in FIG. **7** (1 IU) in three different formulations (i) insulin administered alone, (ii) insulin combined with VSP at a 1:1 ratio, and (iii) insulin combined with VSP at a 1:3 ratio. PBS and a subcutaneous administration of insulin at 1-5 IU were used as controls. The combination of 1 IU of insulin with VSPs enhanced insulin's biological action, at a 1:1 insulin/VSP ratio for LANTUS® and at a 1:3 insulin/VSP ratio for NOVORAPID® (circled).

Example 6

IN VITRO Protection of Human Growth Hormone (hGH) by the VSP1267 VSP Carrier

To evaluate in vitro the capacity of a VSP carrier to protect bioactive peptides from degradation, we evaluated the capacity of recombinant VSP1267 to protect human growth hormone (somatotropin, hGH) (Biosidus, Argentina), a 191 amino acids long protein, from conditions similar to those present in the GIT. Similar to the previous analysis performed with the VSPs alone, the capacity of the VSP to protect hGH from degradation caused by extreme pHs or by enzymatic proteolysis was assayed.

First, the specificity of the αHCB anti-hGH monoclonal antibody was determined by Western blot (FIG. **9**). Two dilutions of the monoclonal antibody αHCB (1/3000 and 1/2000), as well as a control anti-alkaline phosphatase antibody (αMouse-AP1) were used to detect hGH (HCB: human growth hormone produced in transgenic bovines). The αHCB monoclonal antibody recognized only one band of the correct molecular weight of hGH (22.1 KDa). Different amounts of hGH (0.25, 0.5, 1, 5 and 10 μg) were used. The αHCB hGH specific monoclonal antibody was capable of detecting very low amounts of hormone at very high dilutions. The anti-mouse antibody used as control showed no reaction.

The anti-hGH monoclonal antibody was subsequently used to determine the degree of resistance of hGH to different pHs. hGH was incubated at different pHs (1.6, 2.0, 3.8, 5.0, 5.8, 7.0, 8.0, 9.0, 10.0, and 11.0) for 90 minutes. The Western blot results shown in the top panel of FIG. **10**A as well as the silver staining detection of the hormone shown in the bottom panel of FIG. **10**A, indicated that at higher pHs hGH remained unaltered as compared with the control. The hormone suffered auto-proteolytic processing at slightly acidic pHs that did not interfere with the recognition of anti-GH antibody (see, e.g., Such-Sanmartin et al., Growth Factors 27:255-64 (2009)). However, at very low pHs (similar to those found in the stomach) part of the hormone was highly degraded (FIG. **10**A, circles).

When a VSP carrier was added to hGH at a 3:1 VSP/hGH ratio and the mixtures were incubated at different pHs (1.4, 1.96, 3.8, 4.91, 5.9, 7.01, 7.95, 8.51, 9.61 and 11.17), no significant changes respect to the hGH degradation levels of hGH in the absence of VSP were observed (FIG. **10**B).

hGH was also treated for 90 min at 37° C. with several concentrations of trypsin (0, 100, 150, 200, and 500 μg/ml). The Western blot results shown in the top panel of FIG. **11**A as well as the silver staining detection of the hormone shown in the bottom panel of FIG. **11**A indicated that the hormone was rapidly degraded even at the lowest protease concentrations. VSP addition at a 3:1 ratio was able to protect hGH from trypsin degradation (FIG. **11**B). This protective effect was observed up to a 150 μg/ml trypsin concentration.

Example 7

In Vivo Protection of Human Growth Hormone (hGH) Administered in Combination with a VSP Carrier

hGH serum levels were tested after oral administration, evaluating different doses and measurement times, to determine the best dose for combination with the VSP carrier VSP1267 (FIG. **11**A). As in previous experiments, female Balb/c mice, 7 weeks-old, were left without food intake for 2 hours and then received the doses indicated in FIG. **12**A (i.e., 50, 100, 200, 400 and 800 μg of hGH). In a parallel experiment, hGH was administered subcutaneously (FIG. **11**A, inset). It was observed that hGH alone is absorbed orally, at much lower levels than subcutaneously absorption (FIG. **11**A, main panel). However, this oral absorption was highly variable regarding both times and amounts. Thus, a direct relationship between absorption and these variables (time and concentration) could not be determined. Despite these results, we used a dose of 50 μg to evaluate the effect of combination of hGH with the VSP carrier.

FIG. **12** shows a time response drawing showing serum hGH levels in female Balb/c mice, 7 weeks-old, that were left without food intake for 2 hours and then received a 50 n dose of hGH in combination with a VSP carrier. Oral administration of hGH:VSP at a 1:3 ratio (50 μg of hGH combined with 150 μg of VSP) enhanced the hGH absorption when compared to the oral administration of hGH alone without a VSP carrier.

Example 8

IN VITRO and In Vivo Protection of Parathormone in Combination with a VSP Carrier

Parathyroid hormone (PTH), parathormone or parathyrin, is secreted by the chief cells of the parathyroid glands as a polypeptide containing 84 amino acids. It acts to increase the concentration of calcium (Ca^{2+}) in the blood, whereas calcitonin (a hormone produced by the parafollicular cells (C cells) of the thyroid gland) acts to decrease calcium concentration. PTH acts to increase the concentration of calcium in the blood by acting upon the parathyroid hormone 1 receptor (high levels in bone and kidney) and the parathyroid hormone 2 receptor (high levels in the central nervous system, pancreas, testis, and placenta). PTH half-life is approximately 4 minutes. It has a molecular mass of 9.4 kDa. A low level of PTH in the blood is known as hypoparathyroidism. Causes include surgical misadventure (e.g., inadvertent removal during routine thyroid surgery), autoimmune disorder, and inborn errors of metabolism. Hypoparathyroidism can be treated, e.g., with synthetic PTH 1-34 (Tireparatide). PTH can be measured in the blood in several different forms: intact PTH; N-terminal PTH; mid-molecule PTH, and C-terminal PTH, and different tests are used in different clinical situations.

To assess in vitro the capacity of VSP carriers to protect parathormone from degradation, the capacity of a VSP carrier (e.g., VSP carrier VSP1267) to protect human parathormone from conditions similar to those present in the GIT is evaluated. The capacity of the VSP to protect parahormone from degradation caused by extreme pHs or by enzymatic proteolysis (e.g., by trypsin and/or pancreatine) is assayed using the methods described in the Examples above.

Parathormone alone or mixed with VSP carrier is incubated at different pHs or with different concentration of proteolytic enzymes such as trypsin. The presence of parathormone after the pH and proteolytic enzyme challenges is detected using an anti-parathormone monoclonal antibody.

Experimental results will show whether the combination of a VSP carrier with parathormone increases the resistance of parathormone to proteolysis and to pH-induced degradation.

In vivo assays to determine whether oral administration of parathormone in combination with a VSP carrier protects parathormone from the conditions in the GIT and results in increased absorption with respect to parathormone orally administered without a VSP carrier are performed using the methods described in previous Examples. E.g., parathormone serum levels are measured at different times after the oral administration of parathormone (alone or in combination with a VSP carrier) to mice at different doses, and parathromone/VSP ratios. The result will indicate whether absorption of parathormone and parathromone serum levels are increased when parathormone is administered in combination with a VSP carrier as compared to the oral administration of parathormone alone without a VSP carrier.

Example 9

IN VITRO and In Vivo Protection of Interleukin 2 (IL-2) in Combination with a VSP Carrier

In preliminary experiments, VSP-IL-2 fusion proteins were produced in which IL-2 was genetically fused to a VSP. One of these recombinant proteins comprised a VSP C-terminally fused to IL-2. In a second recombinant construct, VSP was C-terminally rased to IL-2 via a linker interposed between the C-terminus of the VSP and the N-terminus of IL-2.

Interleukin-2 (IL-2) is an interleukin, a type of cytokine signaling molecule in the immune system. It is a protein that attracts white blood cells (lymphocytes of leukocyte), the cells that are responsible for immunity. It is part of the body's natural response to microbial infection, and in discriminating between foreign (non-self) and self. IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes. IL-2 has been tested in many clinical trials as an immunotherapy for the treatment of cancers, chronic viral infections and as adjuvants for vaccines. A recombinant form of IL-2 for clinical use is manufactured by Prometheus Laboratories Inc with the brand name Proleukin. It has been approved by the Food and Drag Administration (FDA) for the treatment of cancers (malignant melanoma, renal cell cancer), and is in clinical trials for the treatment of chronic viral infections, and as a booster (adjuvant) for vaccines.

To assess in vitro the capacity of VSP carriers to protect IL-2 from degradation, the capacity of a VSP carrier (e.g., recombinant VSP1267) to protect human IL-2 from conditions similar to those present in the GIT is evaluated. The capacity of the VSP to protect IL-2 from degradation caused by extreme pHs or by enzymatic proteolysis (e.g., by trypsin and/or pancreatine) is assayed using the methods described in the Examples above.

IL-2 alone or mixed with VSP carrier is incubated at different pHs or with different concentration of proteolytic enzymes such as trypsin. The presence of IL-2 after the pH and proteolyitc enzyme challenges is detected using an anti-IL-2 monoclonal antibody. Experimental results will

show whether the combination of a VSP carrier with IL-2 increases the resistance of IL-2 to proteolysis and to pH-induced degradation.

In vivo assays to determine whether oral administration of IL-2 in combination with a VSP carrier protects IL-2 from the conditions in the GIT and results in increased absorption with respect to IL-2 orally administered without a VSP carrier are performed using the methods described in previous Examples. E.g., IL-2 serum levels are measured at different times after the oral administration of IL-2 (alone or in combination with a VSP carrier) to mice at different doses, and IL-2/VSP ratios. Biological effects of IL-2 is assessed by monitoring in regulatory T cells numbers/frequencies as well as expression of CD25 on Treg. The result will indicate whether absorption of IL-2 and (i) IL-2 serum levels are increased, (ii) and/or Treg numbers/frequencies is increased, (iii) and/or CD25 molecule expression detected by mean fluorescent intensity of staining using flow cytometry is increased, when IL-2 is administered in combination with a VSP carrier as compared to the oral administration of IL-2 alone without a VSP carrier.

Example 10

IN VITRO and In Vivo Protection of Interleukin 10 (IL-10) in Combination with a VSP Carrier

Interleukin-10 (IL-10 or IL10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. In humans IL-10 is encoded by the IL10 gene. IL-10 is capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF made by cells such as macrophages and regulatory T-cells. It also displays a potent ability to suppress the antigen-presentation capacity of antigen presenting cells. However, it is also stimulatory towards certain T cells and mast cells and stimulates B cell maturation and antibody production.

To assess in vitro the capacity of VSP carriers to protect IL-10 from degradation, the capacity of a VSP carrier (e.g., recombinant VSP1267) to protect human IL-10 from conditions similar to those present in the GIT is evaluated. The capacity of the VSP to protect IL-10 from degradation caused by extreme pHs or by enzymatic proteolysis (e.g., by trypsin and/or pancreatin) is assayed using the methods described in the Examples above.

IL-10 alone or mixed with VSP carrier is incubated at different pHs or with different concentration of proteolytic enzymes such as trypsin. The presence of IL-10 after the pH and proteolytic enzyme challenges is detected using an anti-IL-10 monoclonal antibody. Experimental results will show whether the combination of a VSP carrier with IL-10 increases the resistance of IL-10 to proteolysis and to pH-induced degradation.

In vivo assays to determine whether oral administration of IL-10 in combination with a VSP carrier protects IL-10 from the conditions in the GIT and results in increased absorption with respect to IL-10 orally administered without a VSP carrier are performed using the methods described in previous Examples. E.g., IL-10 serum levels are measured at different times after the oral administration of IL-10 (alone or in combination with a VSP carrier) to mice at different doses, and IL-10/VSP ratios. The results will indicate whether absorption of IL-10 and IL-10 serum levels are increased when IL-10 is administered in combination with a VSP carrier as compared to the oral administration of IL-10 alone without a VSP carrier.

Example 11

IN VITRO and In Vivo Protection of Glucagon in Combination with a VSP Carrier

Glucagon, a peptide hormone secreted by the pancreas, raises blood glucose levels. Its effect is opposite that of insulin, which lowers blood glucose levels. Glucagon is a 29-amino acid polypeptide. Its primary structure in humans is HSQGTFTSDYSKYLDSRRAQDFVQWLMNT (SEQ ID NO:6). The polypeptide has a molecular weight of 3,485 daltons. Glucagon administration is vital first aid in cases of severe hypoglycemia when the victim is unconscious or for other reasons cannot take glucose orally. Glucagon is given by intramuscular, intravenous or subcutaneous injection, and quickly raises blood glucose levels. The reconstitution process makes using glucagon cumbersome (Meeran et al., Endocrinology 140(1):244-50 (1999); Longuet et al., Cell Metab. 8(5):359-71 (2008)).

To assess in vitro the capacity of VSP carriers to protect glucagon from degradation, the capacity of a VSP carrier (e.g., recombinant VSP1267) to protect human glucagon from conditions similar to those present in the GIT was evaluated. The capacity of the VSP to protect glucagon from degradation caused by enzymatic proteolysis by trypsin was assayed using the methods described in the Examples above.

Glucagon (1 μg) alone or mixed with VSP carrier was incubated with different concentrations of trypsin at 37° C. for 1 hour (FIG. **13**A). The reaction was stopped by addition of protease inhibitor cocktail (Complete, EDTA-free, Roche Diagnostic). The presence of glucagon after the pH and proteolyitc enzyme challenges was detected using an anti-glucagon monoclonal antibody. Experimental results showed that the combination of a VSP carrier with glucagon increased the resistance of glucagon to trypsin-induced proteolysis. The top panel of FIG. **13**A is a dot blot analysis showing that trypsin proteolyzed glucagon. The bottom panel of FIG. **13**A shows that combining a VSP carrier with glucagon at a 1:3 glucagon to VSP ratio protected glucagon from trypsin degradation up to 1:2 (protein:protease) ratio. The Dot blots corresponded to pairs of samples in which glucagon samples without a VSP carrier, or with a VSP carrier at a 1:3 glucagon to VSP carrier ratio were subjected to the same trypsin concentrations.

The glucagon used in these assays was a polypeptide hormone of recombinant DNA origin (r-Glucagon, Lilly) marketed by Eli Lilly Company of Mexico S.A. de C.V. Predictive computer analysis (web.expasy.org/cgi-bin/peptide_cutter/peptidecutter.pl) had indicated that this peptide was sensitive to the action of the enzyme trypsin, as shown in FIG. **13**A. Preliminary tests on the sensitivity of anti-glucagon antibody (Sigma-Aldrich Cat.# G2654) used in the experimental procedures described herein determined that 1 μg of this peptide was detected by the antibody.

FIG. **13**B shows that VSP promotes the biological action of glucagon when glucagon is co-administered orally. Effect of oral administration of glucagon was evaluated in BALC/c mice of 7 weeks of age, which were left without food intake for 2 hs and then received the indicated doses of glucagon alone or combined with VSP. The blood glucose levels were determined at the indicated times. The combination of 50 μg glucagon with VSPs (150 μg) appears to increase the biological action of glucagon when it is administrated orally,

respect to the oral administration of glucagon alone. Moreover, from the in vivo test is remarkable to note that the animals that have received an oral administration of glucagon plus VSP their glucose levels increased more quickly (15 minutes) respect to the animals with subcutaneous (S.C) inoculation (30 minutes), and the effect of the group glucagon-VSP oral was maintained greater amount of time respect to the s.c. group.

In vivo assays to determine whether oral administration of glucagon in combination with a VSP carrier protects glucagon from the conditions in the GIT and results in increased absorption with respect to glucagon orally administered without a VSP carrier were performed using the methods described in previous Examples. Glucagon serum levels were measured at different times after the oral administration of glucagon (alone or in combination with a VSP carrier) to mice at different doses, and glucagon/VSP ratios (FIG. **13**B). In particular, the effect of oral administration of glucagon was evaluated in BALC/c mice of 7 weeks of age, which were left without food intake for 2 hours and then received the indicated doses of glucagon alone or combined with VSP. The blood glucose levels were determined at the indicated times. The combination of 50 μg glucagon with VSPs (150 μg) appeared to increase the biological action of glucagon when it was administrated orally, respect to the oral administration of glucagon alone. Moreover, from the in vivo test is was remarkable to observe that the animals that have received an oral administration of glucagon plus VSP saw their glucose levels increase more quickly (15 minutes) with respect to the animals that underwent subcutaneous (S.C) inoculation (30 minutes). The effect observed on the group receiving glucagon-VSP orally was maintained for greater amount of time with respect to the group receiving the composition subcutaneously.

Example 12

Delivery of Poorly Soluble Small Molecule Drugs in Combination with VSP Carriers

To assess in vitro the capacity of VSP carriers to effectively deliver poorly soluble therapeutic agents (e.g., poorly soluble small molecule drugs such as glipizide, a poorly water-soluble BCS class II antidiabetic drug; amikacin, an aminoglycoside antibiotic; or vancomycin, a glycopeptide antibiotic), the capacity of a VSP carrier (e.g., recombinant VSP1267) to protect the poorly soluble therapeutic agent from conditions similar to those present in the GIT is first evaluated. The capacity of the VSP to protect the poorly soluble therapeutic agent from degradation caused by extreme pHs is assayed using methods known in the art. For example, the therapeutic agent in combination with a VSP carrier is subjected the pH levels similar to those present in the GIT, and the degradation of the drug is monitored using mass spectrometry or chromatographic methods such as HPLC. Additionally, the capacity of the VSP to solubilize the therapeutic can be measured by methods known in the art. Experimental results will show whether the combination of a VSP carrier with the poorly soluble therapeutic agent increases the therapeutic agent's solubility while protecting it from degradation by conditions similar to those present in the GIT.

In vivo assays to determine whether oral administration of the poorly soluble therapeutic agent in combination with a VSP carrier effectively keeps the therapeutic agent in solution, protects the therapeutic agent from the conditions in the GIT, and results in increased absorption with respect to the therapeutic agent administered without a VSP carrier are performed using the methods described in previous Examples and methods known in the art. The results will indicate whether combining the therapeutic agent with a VSP can maintain the therapeutic agent in solution, protect in from the conditions in the GIT, and increase the absorption of the therapeutic agent and its serum levels when compared to the oral administration of poorly soluble therapeutic agent alone without a VSP carrier.

The present invention has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US09457096B2). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A therapeutic composition comprising a VSP (Variant-specific Surface Protein) carrier and a bioactive peptide, wherein

(i) the VSP carrier can bind to the bioactive peptide;

(ii) the VSP carrier is not covalently bound to the bioactive peptide via a peptidic bond;

(iii) the bioactive peptide is therapeutically effective after binding to the VSP carrier;

(iv) the binding of the VSP carrier to the bioactive peptide increases the resistance of the bioactive peptide to pH-mediated and/or enzymatic degradation compared to the resistance of the same bioactive peptide not bound to said VSP carrier;

(v) the bioactive peptide is selected from the group consisting of insulin, human growth hormone, and glucagon;

(vi) the bioactive peptide is not a vaccine immunogen, and

(vii) the VSP carrier has at least 70% amino acid sequence identity with the sequence of the extracellular domain of a VSP from *Giardia* selected from VSP1267 (SEQ ID NO: 490), VSP9B10 (SEQ ID NO: 572), and VSPH7 (SEQ ID NO:504).

2. The composition of claim 1, formulated for oral administration.

3. The composition of claim 1, formulated for mucosal administration.

4. The composition of claim 1, wherein the VSP carrier further comprise a protein purification tag sequence.

5. The composition of claim 4, wherein the protein purification tag sequence is a His6 tag.

6. The composition of claim 1, wherein the VSP carrier consists of the sequence of SEQ ID NO:1.

7. The composition of claim 1, wherein the insulin is a natural insulin.

8. The composition of claim 1, wherein the insulin is a recombinant insulin.

9. The composition of claim 1, wherein the insulin is an insulin analog.

10. The composition of claim 9, wherein the insulin analog is a fast-acting insulin.

11. The composition of claim 9, wherein the insulin analog is a long-acting insulin.

12. The composition of claim 10, wherein the fast-acting insulin is insulin aspart.

13. The composition of claim 11, wherein the long-acting insulin is insulin glargine.

14. The composition of claim 1, wherein the molecule to molecule ratio of VSP carrier to the bioactive peptide ranges from about 10:1 to about 1:10.

15. The composition of claim 14, wherein the molecule to molecule ratio of VSP carrier to the bioactive peptide ranges from about 3:1 to about 1:3.

16. The composition of claim 15, wherein the molecule to molecule ratio of VSP carrier to the bioactive peptide is 3:1.

17. The composition of claim 15, wherein the molecule to molecule ratio of VSP carrier to the bioactive peptide is 1:1.

18. The composition of claim 1, further comprising a pharmaceutically acceptable excipient.

19. A method of delivering a bioactive peptide to a target location in a subject comprising administering the therapeutic composition of claim 1 to a subject in need thereof.

20. A method of treating a disease or condition in a subject comprising administering an effective amount of the therapeutic composition of claim 1 to a subject in need thereof, wherein the disease or condition is a hormone deficiency.

21. The method of claim 20, wherein the hormone deficiency is an insulin deficiency.

22. The method of claim 21, wherein the insulin deficiency is type 1 diabetes.

23. A method of treating a disease or condition in a subject comprising (a) combining a VSP carrier and a bioactive peptide, wherein the VSP carrier can bind to the bioactive peptide, wherein the bioactive peptide is not a vaccine immunogen, therein the VSP carrier is not covalently bound to the bioactive peptide via peptidic bonds, wherein the bioactive peptide is therapeutically effective after binding to the VSP carrier, the binding of the VSP carrier to the bioactive peptide increases the resistance of the bioactive peptide to pH-mediated and/or enzymatic degradation compared to the resistance of the same bioactive peptide not bound to said VSP carrier, wherein the bioactive peptide is selected from the group consisting of insulin, human growth hormone, and glucagon, and wherein the VSP carrier has at least 70% amino acid sequence identity with the sequence of the extracellular domain of a VSP from *Giardia* selected from VSP1267 (SEQ ID NO:490), VSP9B10 (SEQ ID NO:572), and VSPH7 (SEQ ID NO:504); and wherein administering an effective amount of the combination of said VSP carrier and bioactive peptide treats the disease or condition in the subject, wherein the disease or condition is a hormone deficiency.

24. A method of making an orally deliverable composition, comprising combining a VSP carrier and a bioactive peptide, wherein the VSP carrier can bind to the bioactive peptide, wherein the bioactive peptide is not a vaccine immunogen, therein the VSP carrier is not covalently bound to the bioactive peptide via peptidic bonds, the binding of the VSP carrier to the bioactive peptide increases the resistance of the bioactive peptide to pH-mediated and/or enzymatic degradation compared to the resistance of the same bioactive peptide not bound to said VSP carrier, wherein the bioactive peptide is therapeutically effective after binding to the VSP carrier, wherein the bioactive peptide is selected from the group consisting of insulin, human growth hormone, and glucagon, and the VSP carrier has at least 70% amino acid sequence identity with the sequence of the extracellular domain of a VSP from *Giardia* selected from VSP1267 (SEQ ID NO:490), VSP9B10 (SEQ ID NO:572), and VSPH7 (SEQ ID NO:504).

25. A method of making an injectable composition suitable for oral administration comprising combining a VSP carrier and a bioactive peptide, wherein the VSP carrier can bind to the bioactive peptide, wherein the bioactive peptide is not a vaccine immunogen, wherein the VSP carrier is not covalently bound to the bioactive peptide via peptidic bonds, the binding of the VSP carrier to the bioactive peptide increases the resistance of the bioactive peptide to pH-mediated and/or enzymatic degradation compared to the resistance of the same bioactive peptide not bound to said VSP carrier, thereby making the injectable composition suitable for oral administration, wherein the bioactive peptide is therapeutically effective after binding to the VSP carrier, wherein the bioactive peptide is selected from the group consisting of insulin, human growth hormone, and glucagon, and the VSP carrier has at least 70% amino acid sequence identity with the sequence of the extracellular domain of a VSP from *Giardia* selected from VSP1267 (SEQ ID NO:490), VSP9B10 (SEQ ID NO: 572), and VSPH7 (SEQ ID NO: 504).

26. (Withdrawn-previously presented) The method of claim 25, wherein the VSP carrier further comprises a protein purification tag sequence.

27. The method of claim 26, wherein the protein purification tag sequence is a His6 tag.

28. The method of claim 25, wherein the VSP carrier consists of the sequence of SEQ ID NO:1.

29. The method of claim 28, wherein the insulin is a natural insulin.

30. The method of claim 28, wherein the insulin is a recombinant insulin.

31. The method of claim 28, wherein the insulin is an insulin analog.

32. The method of claim 31, wherein the insulin analog is a fast-acting insulin.

33. The method of claims 31, wherein the insulin analog is a long-acting insulin.

34. The method of claim 32, wherein the fast-acting insulin is insulin aspart.

35. The method of claim 33, wherein the long-acting insulin is insulin glargine.

36. The composition of claim 1, wherein the VSP carrier having at least 70% amino acid sequence identity with the sequence of the extracellular domain of VSP1267 (SEQ ID NO: 490), VSP9B10 (SEQ ID NO:572), or VSPH7 (SEQ ID NO: 504) is selected from SEQ ID NO: 58, 143, 163, 166, 167, 308, and 486, or a fragment thereof.

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